

Formulation of Antiaging Gel from Roselle Calyces Extract

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Abstract: Roselle (*Hibiscus sabdariffa* L.) is known to have many active compounds that function as antioxidants such as flavonoids, polyphenols, and vitamin C. High antioxidant content has a high antiaging effect. This study aims to create a cream dosage form that has antiaging properties with active ingredients roselle calyx ethanolic extract that meets the requirements of physical evaluation, stability evaluation, and has an antiaging activity before and after use. The ethanol extract of roselle calyx is made by maceration. Creams were made with variations of vitamin C concentration of 1% (w/w), and 2.0% (w/w), also roselle calyx ethanolic extract concentration of 0.5% (w/w), 1% (w/w), 1.5% (w/w), and 2.0% (w/w). Antiaging activity test performed by measurement of moisture, evenness, pore, spot, and wrinkle by using skin analyzer tool. The results showed that the antiaging cream of roselle calyx ethanolic extract has a good physical properties and good stability properties for 1 month of storage. All creams have significantly different antiaging activities and depend on the roselle calyx ethanolic extract concentration. The higher concentration of roselle calyx ethanolic extract, the higher antiaging activity. Antiaging activity with a concentration of roselle calyx ethanolic extract had no significant difference with vitamin C.

Keywords: Antiaging, Cream, Roselle Calyx, *Hibiscus sabdariffa*, Vitamin C

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I. INTRODUCTION

Skin is the body's main protector from exposure to free radicals that come from outside the body. These free radicals can be UV rays, air pollution, dust, and exposure to exogenous chemicals (Wahyuni, 2005). Free radicals can damage the collagen and elastin structures of the skin, causing the skin to become less elastic and wrinkle lines, disturbing the distribution of melanin pigment and melanocytes resulting in uneven pigmentation, damaging the macrobuilding molecules of proteins, carbohydrates, fats, and can cause skin cancer (Jusuf, 2005). Free radical effects can be prevented by the use of antioxidants. Roselle (*Hibiscus sabdariffa* L.) is a medicinal plant that has the property to prevent premature aging (antiaging) because of the antioxidant content. The major bioactive compounds that act as antioxidants are flavonoids, polyphenols, and ascorbic acid (Sarhini, 2007). The use of roselle as a skin protective is still rarely used by the community, so it needs to be developed into a form of topical dosage form of cream. Cream was one of the favorite preparation because of its properties are easy to apply, easy to wash with water, not sticky, and can provide a sense of comfort for the user because it contains high water content so as to maintain skin moisture and elasticity (Mitsui, 1997). Based on these descriptions, a study was conducted to determine the physical properties and stability properties of the antiaging cream by using roselle calyx ethanolic extract and the antiaging activity of the preparation using skin analyzer tool.

II. MATERIALS AND METHODS

Materials

Materials used in this research was: roselle calyx from Berastagi, North Sumatera, stearic acid, stearyl alcohol, cetyl alcohol, potassium hydroxide, methyl paraben, propyl paraben, glycerin, strawberry flavour, water, isopropanol, ethanol 70 %, ethanol 96%.

Tools

Tools used in this research was: skin analyzer (Aramo SG), analytical balance (Shimadzu), pH meter (Hanna), UV lamp, rotary evaporator, freeze dryer, guinea pig guard cage, drying cabinet, laboratory glassware, water bath, mortar, pestle, plastic pot, scissors and guinea pig shavers

Preparation of Extract

Preparation of roselle calyx ethanolic extract was done dried 1000 grams of roselle calyx, 300 grams of dried roselle calyx was macerated in 1.5 L ethanol 70% for 3 × 24 hours, then filtered using Buchner funnel. The extract was then evaporated using rotary evaporator at 40°C (Anokwuru et al., 2011).

Preparation of Cream Dosage Form

Cream dosage form is made in 7 formulas. One formulas without roselle calyx ethanolic extract and vitamin C. Two formulas with variation of vitamin C level. Each cream contains a vitamin C level 1.0% w/w, and 2.0% w/w in the same cream base composition. Four formulas with variation of roselle calyx ethanolic extract level. Each cream contains a roselle calyx ethanolic extract level 0.5% w/w, 1.0% w/w, 1.5% w/w, and 2.0% w/w in the same cream base composition. The composition of the cream formula can be seen in Table 1.

Table 1. Composition of various cream formula

COMPOUND	F1	F2	F3	F4	F5	F6	F7
Vitamin C (g)	-	1,0	2,0	-	-	-	-
Roselle Calyx Ethanolic Extract (g)		-	-	0,5	1,0	1,5	2,0
Stearic Acid (g)	13,0	13,0	13,0	13,0	13,0	13,0	13,0
Cetyl Alcohol (g)	1,0	1,0	1,0	1,0	1,0	1,0	1,0
Stearyl Alcohol (g)	1,0	1,0	1,0	1,0	1,0	1,0	1,0
Methyl paraben (g)	0,1	0,1	0,1	0,1	0,1	0,1	0,1
Propyl paraben (g)	0,05	0,05	0,05	0,05	0,05	0,05	0,05
Glycerin (g)	10,0	10,0	10,0	10,0	10,0	10,0	10,0
Potassium Hydroxide (g)	0,9	0,9	0,9	0,9	0,9	0,9	0,9
Strawberry Flavour (g)	0,1	0,1	0,1	0,1	0,1	0,1	0,1
Water (Ad)	100	100	100	100	100	100	100

The creams are prepared by separately heated the oil phase (stearic acid, stearyl alcohol, propyl paraben and cetyl alcohol) and water phase (water, potassium hydroxide, glycerin and methyl paraben) at 70°C. The water phase is mixed into the oil phase inside the hot mortar and then stirred strongly for 10 minutes until the cream base is formed. Once the base is formed, the extract or vitamin C is mixed into the base of the cream, then stirring slowly, adding sufficient strawberry odors, then cooled.

Evaluation of Physical Properties and Stability Properties of Antiaging Cream

Cream physical properties and stability properties was evaluated for 4 weeks which included organoleptic observation (color change and odor change) and pH measurement.

Antiaging Test

The test animals used in this study were 21 guinea pig with weight 350-500 grams. Guinea pig was shaved its back hair covering an area of 2.5 cm × 2.5 cm, 1 day before irradiation. Then the guinea pig is divided into 7 groups, namely:

- Group I :Blank cream (without active ingredients)
- Group II: Antiaging cream with vitamin C was 1.0%
- Group III: Antiaging cream with vitamin C was 2.0%
- Group IV: Antiaging cream with roselle calyx ethanolic extract level 0.5%
- Group V: Antiaging cream with roselle calyx ethanolic extract level 1.0%
- Group VI: Antiaging cream with roselle calyx ethanolic extract level 1.5%
- Group VII: Antiaging cream with roselle calyx ethanolic extract level 2.0%

Prepared cage guinea pig guard to be irradiated. A total of 21 guinea pigs first shaved the back of the fur size of 2.5 cm × 2.5 cm. Measured skin condition (moisture, evenness, pore, spot, and wrinkle) before irradiation. Then exposed to UV light with a wavelength of 366 nm for ± 5 hours to form wrinkles. Further measured skin conditions (moisture, evenness, pore, spot, and wrinkle) after irradiation. Once the skin is obtained the wrinkle then recovery can be started. Polishing cream in accordance with the division of each group on the skin guinea pig 2 times a day evenly on the area shaved. Then performed the skin condition (moisture, evenness, pore, spot, and wrinkle) every week for 4 weeks using skin analyzer. Observe skin conditions (moisture, evenness, pore, spot, and wrinkle) of guinea pigs of each group before and after giving cream.

III. RESULTS AND DISCUSSIONS

Preparation of Extract

From 1000 grams of roselle calyx obtained 454,64 grams of dried roselle calyx. From 300 grams of dried roselle calyx obtained 75,69 grams roselle calyx ethanolic extract.

Preparation of Cream Dosage Form

The antiaging cream obtained was good physical properties and will be conducted evaluation of physical properties and stability properties of antiaging cream, also conducted for antiaging test.

Evaluation of Physical Properties and Stability Properties of Antiaging Cream

Cream physical properties and stability properties was evaluated for 4 weeks which included organoleptic observation (color change and odor change) and pH measurement. Table 2 showed the evaluation of physical properties and stability properties of antiaging cream results.

Table 2. Evaluation of physical properties and stability properties of antiaging cream results

FORMULA	WEEK			
	1	2	3	4
F1	A (-) B (-) pH 6.2	A (-) B (-) pH 6.2	A (-) B (-) pH 6.2	A (-) B (-) pH 6.1
F2	A (-) B (-) pH 6.0	A (-) B (-) pH 5.9	A (-) B (-) pH 5.9	A (-) B (-) pH 5.8
F3	A (-) B (-) pH 5,6	A (-) B (-) pH 5.5	A (-) B (-) pH 5.5	A (-) B (-) pH 5.4
F4	A (-) B (-) pH 5,3	A (-) B (-) pH 5.3	A (-) B (-) pH 5.2	A (-) B (-) pH 5.2
F5	A (-) B (-) pH 5,1	A (-) B (-) pH 5.1	A (-) B (-) pH 5.0	A (-) B (-) pH 5.0
F6	A (-) B (-) pH 5,7	A (-) B (-) pH 5.6	A (-) B (-) pH 5.6	A (-) B (-) pH 5.5
F7	A (-) B (-) pH 5,4	A (-) B (-) pH 5.4	A (-) B (-) pH 5.3	A (-) B (-) pH 5.3

A : Color change; B : Odor Change

Ascorbic acid is stable in a dry state, but it is easily oxidized in solution ie ascorbic acid oxidized to dehydroascorbic acid (Kusharto and Suhardjo, 1992). While anthocyanins if dissolved in a mixture will oxidize slowly. The rate of anthocyanin degradation tends to increase during the storage process, accompanied by a rise in room temperature. Thermal degradation causes the color loss of anthocyanin that eventually browns (Hayati, 2012).

Stability test data showed that the cream preparation was stable for 4 weeks storage. The resulting cream does not show the color change and odor change, good homogeneity. From the results of stability observations it was found that without the addition of antioxidants it has been able to obtain a stable cream in 4 weeks storage. Damage or failure of a preparation containing oxidizable substances can be observed in the presence of color changes and odor changes (Ansel, 1989).

Antiaging Test

Measured ginea pig skin condition after shaved guinea pig backhair of the fur size of 2.5 cm × 2.5 cm. Then exposed to UV light with a wavelength of 366 nm for ± 5 hours to form wrinkles. Further measured skin condition after irradiation. Once the skin is obtained the wrinkle then recovery can be started. Polishing cream in accordance with the division of each group on the skin guinea pig 2 times a day evenly on the area shaved. Then the skin condition is measured every week for 4 weeks using a skin analyzer. Observe the guinea pig skin condition of each group before and after the cream. Table 3 showed the antiaging test of antiaging cream results.

Table 3. Antiaging test of antiaging cream results

FORMULA		Results					
		Before Irradiation	After Irradiation	Week 1 Recovery	Week 2 Recovery	Week 3 Recovery	Week 4 Recovery
F1	M	31.33±0.57	13.33±0.57	15.67±1.15	17.67±1.15	20.33±0.57	23.00±0.00
	E	49.67±1.15	61.33±1.52	59.67±3.05	58.33±2.64	56.67±0.57	55.33±0.57
	P	29.33±4.72	68.67±1.05	65.67±1.65	61.33±1.25	58.33±3.63	54.67±2.71
	S	27.67±4.07	68.33±1.34	64.33±1.93	60.67±1.26	53.33±1.01	46.33±2.07
	W	5.00±0.00	61.33±1.79	50.66±1.85	39.33±0.57	37.00±0.00	35.33±4.92
F2	M	33.33±1.03	14.33±1.75	19.67±1.38	22.00±1.00	25.67±1.35	28.67±1.88
	E	49.33±2.77	62.33±1.26	57.67±1.38	52.67±1.37	48.00±2.00	45.67±1.99
	P	27.33±1.39	66.67±3.16	59.33±1.72	49.33±2.39	43.67±1.58	38.33±3.82
	S	26.00±1.00	66.33±3.51	57.67±2.97	46.33±2.63	30.33±2.54	19.67±3.75
	W	5.00±0.00	63.33±2.46	46.67±3.37	30.33±1.36	21.33±2.26	12.33±3.17
F3	M	33.33±1.55	14.33±0.75	26.67±1.94	30.67±2.16	34.00±1.00	39.00±0.00

	E	47.33 ±1.83	62.67±0.28	58.33±0.81	51.33±1.64	42.67±1.38	36.33±1.79
	P	28.33±1.51	67.00±2.00	56.33±1.62	45.67±3.27	26.67±1.64	18.33±3.02
	S	27.33±4.72	66.33±3.42	50.67±2.17	36.67±1.63	26.67±3.53	16.33±2.61
	W	5.00±0.00	64.33±3.27	46.33±2.27	29.00±3.00	20.33±2.62	11.33±1.28
F4	M	32.67±1.15	14.67±0.57	16.67±0.57	18.33±1.15	20.33±2.88	23.67±3.51
	E	49.33±3.07	62.00±1.00	60.33±3.16	58.67±3.33	56.33±0.63	54.67±1.82
	P	26.00±2.00	67.33±5.50	59.33±4.58	51.33±5.03	47.33±1.15	43.67±5.19
	S	26.33±2.08	67.33±5.03	57.67±4.72	47.67±5.03	40.67±8.50	32.67±5.85
F5	W	5.00±0.00	64.33±1.71	49.67±1.78	40.33±5.72	31.33±6.45	26.67±2.56
	M	33.67±1.22	14.33±1.38	19.33±2.17	22.67±1.88	25.00±0.00	28.33±2.15
	E	48.67±1.77	62.00±1.00	57.33±2.51	52.33±1.52	48.67±2.46	45.33±1.23
	P	27.00±2.00	67.67±2.65	58.33±2.51	50.00±1.00	42.33±3.51	37.00±3.00
F6	S	26.33±1.15	67.67±3.11	56.33±3.05	45.33±4.38	31.33±1.04	18.33±4.65
	W	5.00±0.00	63.67±1.93	47.33±2.69	30.33±3.76	21.67±2.34	12.33±5.67
	M	33.33±0.57	14.33±1.15	23.33±4.00	27.67±2.51	31.67±0.57	34.00±0.00
	E	47.67±1.67	60.67±1.33	56.67±1.47	51.33±1.67	46.33±1.84	40.33±1.33
F7	P	28.33±1.72	67.00±3.00	58.33±2.51	50.00±1.00	42.33±3.51	37.00±3.00
	S	27.33±2.46	66.67±3.12	53.67±1.48	41.64±3.27	28.67±4.62	17.33±2.31
	W	5.00±0.00	64.33±2.72	46.33±2.63	29.33±2.64	20.33±2.26	12.00±1.00
	M	33.00±1.00	14.67±0.86	27.33±2.94	30.33±1.22	34.33±2.15	38.67±1.95
	E	46.67 ±1.31	61.33±0.57	56.33±2.07	50.00±2.00	43.33±1.13	36.67±2.76
	P	28.66±1.15	67.67±1.59	55.33±1.15	44.33±4.72	26.33±2.76	18.33±5.29
	S	27.67±4.72	66.00±3.00	51.33±3.18	37.67±2.56	25.33±5.85	15.67±3.21
	W	5.00±0.00	64.67±3.77	46.67±2.08	29.67±3.11	20.67±2.86	11.67±1.51

M : Moisture (0-29 : Dehydration; 30-50 : Normal; 51-100 : Hydration)

E : Evenness (0-31 : Smooth Skin; 32-51 : Normal Skin; 52-100 : Rough Skin)

P : Pore (0-19 : Small; 20-39 : Medium; 40-100 : Big)

S : Spot (0-19 : Little; 20-39 : Medium; 40-100 : Many)

W : Wrinkle (0-19 : No Wrinkles; 20-52 : Wrinkles; 53-100 : Severe Wrinkles)

The skin is constantly exposed to free radicals caused by UV radiation causing damage to DNA and protein resulting in premature aging of skin cells. When exposed to UV radiation, the skin undergoes changes that lead to inflammation, photoaging, and various skin disorders, aging skin accompanied by wrinkles, decreased elasticity, increased skin fragility, and slower wound healing (Pouillot, et al., 2011). Excessive sun exposure is one factor causing decreased production of collagen in the dermis of the skin, because excessive exposure to sunlight causes the emergence of proteolysis enzymes from free radicals are formed. This enzyme will further damage the skin, destroy collagen, and connective tissue that exist under the skin of the dermis. So that excessive exposure to UV light causes the aging process on the skin takes place more quickly. Premature aging is caused by internal factors (such as genetics, poor energy intake) and external factors (such as pollution, cigarettes, sunlight and the effects of unhealthy lifestyles) (Muliyanawan and Suriana, 2013).

The result of measurement shows that after irradiation there is decreasing of skin moisture, decreasing of skin evenness, increasing of skin spot, increasing of skin pore, increasing of skin wrinkle. In the recovery period, all groups showed improvement in skin with increasing of skin moisture, increasing of skin evenness, decreasing of skin spot, decreasing of skin pore, decreasing of skin wrinkle. It can be known that blank cream also gives recovery effect to skin. However, the blank cream in the use of 4 weeks can not restore the skin to normal. But with the addition of vitamin C and roselle calyx ethanolic extract to cream base (blank cream) then found skin repair in 4 weeks the same as the skin of the norm (before irradiation).

The statistical test using one way analysis of variance showed a significant difference ($p > 0.05$) between groups before irradiation with after irradiation, whereas the recovery of the first week to fourth week found significant difference ($p < 0.05$). Tukey analysis showed a significant difference ($p < 0.05$), in the second week between the group of blank cream treatment, vitamin C cream treatment group, and group with roselle calyx ethanolic extract cream treatment. Recovery every week there are significant differences. By using one way analysis of variance, there was no significant difference between antiaging cream and roselle calyx ethanolic extract 1.0% concentration, and antiaging cream with 1.0% vitamin C concentration, and no significant difference between antiaging cream and roselle calyx ethanolic extract 2.0 concentration %, and antiaging cream with vitamin C concentration of 2.0%. Roselle calyx ethanolic extract has equal antiaging activity to vitamin C in antiaging testing during a 4week recovery.

IV. CONCLUSIONS

Rosellecalyx ethanolic extract can be formulated in a cream preparation, having a pH of 5.0 to 6.0. The resulting cream preparations are all homogeneous. The resulting cream is stable for up to 4 weeks of storage. Roselle calyx ethanolic extract has equal antiaging activity to vitamin C in antiaging testing during a 4week recovery.

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