

# Chlorella Vulgaris Extract Cream as A Formulation of Bone Remodelling

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## ABSTRACT

*Chlorella vulgaris* is one of the green microalgae that has many contents that are beneficial to humans. The bioactive component of microalgae has antimicrobial, antitumor, antioxidant, antiviral, antifungal, and anti-inflammatory activities. Moreover, these bioactive components can produce collagen, a protein that will support cell regeneration, for example, in bone remodeling. One way to increase the effectiveness of using *C. vulgaris* in the bone remodeling process is to formulate *C. vulgaris* extract in cream form. The cream is a semi-solid preparation in the form of an emulsion containing one or more drug ingredients dissolved or dispersed in a suitable base material and contains no less than 60% water. The purpose of this study was to determine the exact formulation of *C. vulgaris* cream extract and to measure the effect of *C. vulgaris* cream extract on the oral mucosa of test animals. Our research is implementing organoleptic, homogeneity, spreadability, viscosity, pH, viscosity, flow type, centrifugation, and irritation tests. The results demonstrated that cream with a concentration of 15% was better than other levels. Cream of 5%, 10%, and 15% concentration did not irritate the mucosa of the test animals. We concluded that the cream with the range concentration did not provoke any oral mucosa adverse reaction of the test animals based.

**Keywords:** Bone remodeling; *Chlorella vulgaris*; Formulation; Irritation

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## INTRODUCTION

Chlorella is a genus of microalgae or single cell of green algae that live in freshwater, the sea, and wet areas. Chlorella comes from the Greek word, chloros, which has meaning in green, and from the Latin word it's called ella, which shows its microscopic size. *Chlorella vulgaris* is one of the green microalgae which have many benefits for human beings. The bioactive component of microalgae has an antimicrobial, antitumor, antioxidant, antiviral, antifungal, and anti-inflammatory function. These bioactive components can produce collagen; the existing protein will support the cell regeneration process, for example, in bone remodeling.<sup>1-3</sup>

The remodeling process is the two stages of cellular activity that occur cyclically, which the resorption of old bone occurred by osteoclasts and new bone formation occurred by osteoblasts. Osteoclasts will carry out resorption through the process of acidification and proteolytic digestion. As soon as osteoclasts leave the resorption area, osteoblasts invade the space and begin the formation process by secreting osteoids (matrix collagen and other proteins), which then undergo mineralization. Typically, the rate of resorption and bone formation takes place at the same speed so that the bone mass remains constant and begins the formation process by secreting osteoid (matrix collagen and other proteins), which then undergoes mineralization. The rate of resorption and bone formation takes place at the same speed so that the bone mass remains constant.<sup>4-6</sup>

The main compositions of *C. vulgaris* are protein, lipid, carbohydrate, pigment, minerals, and vitamins. Mineral content, such as calcium and iron and vitamin D, plays a significant role in bone mineralization, teeth, and

regulation of blood calcium and phosphorus levels.<sup>2</sup>

The way to increase the effectiveness of using the *Chlorella vulgaris* in the bone remodeling process is formulating *Chlorella vulgaris* extract in a cream preparation. The cream is a semi-solid preparation in the form of an emulsion containing one or more ingredients that dissolved or dispersed in an appropriate base material and contained no less than 60% water. Based on the explanation above, the researchers wanted to prove the effect of chlorella *Vulgaris* cream extract, which is safe to use in the bone remodeling process.

## MATERIALS AND METHODS

This research was an experimental laboratory study that used the organoleptic method, spreadability, PH, viscosity, rheology, homogeneity, and centrifugation. The research was conducted at the Pharmaceutical Laboratory and Biopharmacy Laboratory of Pharmacy Department, Hasanuddin University Makassar, between August to September 2018. Analytical scales, porcelain cups, horn spoons, micropipettes, electric stoves, stemper, stirring rods, beakers, aluminum foil, homogenizer, stationery, viscometer, digital calipers, scales, electronic centrifuges, digital pH meters, cotton buds, jars, Eppendorf tubes were used for the experimentation. Mixed ingredients of dried *Chlorella Vulgaris* on 5%, 10%, 15% concentration, stearic acid 5%, cetyl alcohol 2%, sterile alcohol 1%, propylene glycol 10%, glycerol 5%, isopropyl mystic 3%, methylparaben 0.12%, propylparaben 0.018%, emulsifying paraben 10%, distilled water 100 grams. *C. Vulgaris* extract cream was prepared in 5%, 10%, and 15% concentration. Five, 10, and 15 gram of *C. Vulgaris* were mixed with stearic acid 5%, cetyl alcohol 2%,

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stearyl alcohol 1%, propylene glycol 10%, glycerol 5%, isopropyl myristate 3%, methylparaben 0.12%, propylparaben 0.018%, emulsifying 10% wax, and 100 ml distilled water. The amount of each ingredient was calculated according to the melting phase of the water and oil up to a temperature of 70°C. The water phase subsequently pours into the oil phase and homogenizes until the cream base is formed. *C. vulgaris* the added slowly using the homogenizer.

### *Organoleptic test*

Organoleptic tests applying visual observation where shapes, colors, and odors were visually characterized. Cream specifications are supposed to have a soft consistency, homogeneous color and conveyed a pleasant smell.

### *Spreadability test*

The spread test was performed to justify the powder spreading when applied to the skin. More power spreading indicated better physical—the spreading involving a clear glass with a 2 mm thickness and weighing the gram.

### *pH test*

The pH test aimed to determine prepared cream acidity or. The pH condition must be adjusted similar to surrounding tissues such as teeth and gums. The level of acidity or an acceptable pH in the mouth area is between 6-7.

### *Viscosity test*

Viscosity testing aimed to measure cream prepared flow. Higher viscosity means a greater period to produce. The viscosity test was carried out using a Brookfield viscometer and spindle no.7. The 5% of 100 ml *Chlorella Vulgaris* extract cream was put into a glass container then the fixed spindle lowered until its boundary immersed into the cream. Fifty rpm was chosen as a standard.

### *Flowability/rheology test*

This test is carried out by using a Brookfield viscometer set to 5, 10, 20.50, and 100 rpm. The shear stress (F) was calculated using the formulation of  $F = \text{viscosity} \times G$ . Where G is the shear rate or rate of shear obtained through the formula  $G = \text{rpm} \times 1703$ .

### *Homogeneity test*

Homogeneity testing carried out to justify whether the materials used in the product have been mixed or not evenly distributed. The 5% *Chlorella Vulgaris* extract cream was applied to a clean, dry slide to form a thin layer, then covered with a cover glass. The cream is declared homogeneous if observed using a microscope, the cream has a texture that looks like a flat and does not have a clot.

### *Centrifugation test*

This test was performed to observe the presence or absence of phase changes in the preparation and determine the stability of the preparation after massive shaking. Observation of the mechanical test or centrifugation test is one indicator of the physical stability of the cream. A sample of 5% *Chlorella Vulgaris*

extract cream was centrifuged with an automatic centrifuge at a 3000-rpm speed for 5 minutes.

Evaluation of the irritation test of *Chlorella Vulgaris* gel extract gel was carried out using the method of CFTA, using three rats at each concentration of the preparation. The mice used in this study were 120 grams of Wistar white rats. Before treatment, mice were adapted to the environment and food for seven days. Test animals are given food and drink every day. Test animals are also marked using food coloring in a different color for each concentration. The test was carried out for six days.

On day 1, pour the ether in a cotton bag and put it into a clear jar as an anesthetic. After that, insert the rat that has been marked with color into the pot. Observe until the rat passes out. Then the rat is removed from the pot, then the rat's mouth is opened and documented to see the normal state of the oral mucosa. The results of the observations are then recorded. On day 2, pour the ether in a cotton bag and put it into a clear jar as an anesthetic. After that, put the rat that has been marked with color into the jar. Observe until the rat passes away. Then the rat is lifted from the jar, then the rat's mouth is opened. Apply 10% *Chlorella Vulgaris* extract cream as much. 0.25 gr in the labial mucosa and gingival teeth of the RB incisor. Apply using a cotton bud. The rat is then returned to the cage. On day 3, pour the ether in a cotton bag and then put it into a clear jar as an anesthetic. After that, insert the rat that has been marked with color into the jar. Observe until the rat passes out. Then lifted from the jar, then the rat's mouth is opened. Observe the state of the mucosa after basting on day 2. Results from observations are then recorded. Reapply the cream extract of *Chlorella Vulgaris* 10% as much as 0.25 grams on the labial mucosa and gingival teeth of the RB incisors. Apply using a cotton bud. The rat was put back to the cage. On day 4, pour the ether in a cotton bag and put it into a clear jar as an anesthetic. After that, insert the rat that has been marked with color into the pot. Observe until the rat passes out. Then the rat is lifted from the jar, then opened the rat's mouth. Observe the state of the mucosa after applying it on day 3. Results from observations were then recorded. Reapply the cream extract of *Chlorella Vulgaris* 10% as much as 0.25 grams on the labial mucosa and gingival teeth of the incisors mandibula. Apply using a cotton bud. The rat was then returned to the cage. The last observation was made on day 5. Pour ether in a cotton bag and then put it into a clear jar as an anesthetic. After that, insert the rat that has been marked with color into the pot. Observe until the rat passes out. Then the rat is lifted from the jar, then the rat's mouth is opened. Observe the state of the mucosa after basting on day 4. Results from observations were recorded.

## RESULTS

The spread test is performed to show the ability of the cream to spread at the location of use and determine the softness of the cream preparation. From the test data, it can be seen that the 5% concentration cream has the highest spreadability, with a spreading capacity of 149.75 mm<sup>2</sup> or 2.995 cm. The 10% concentration cream has a spreading ability of 2.906 cm, and the 15% concentration cream has a scattering power of 2.192 cm. Cream with a concentration of 10% has the highest pH of

8.66. Then cream with a concentration of 15% has a pH of 8.52, and cream with a concentration of 5% has a pH of 8.35.

From the average test with a speed of 50 rpm, the viscosity of the 5% cream is 17.600 Pa.s (1.760cps) the 10% cream is 20.533 Pa.s (2.053 cups) while 15% is 32.266 Pa.s (3.226 cups).

Graph 4 demonstrated that the 5% concentration cream has a Non-Newtonian flow, which means that a liquid does not show a linear relationship between the change in force (shearing stress) and its viscosity. The curve shows the mean curve; the type of flow is pseudoplastic. A pseudoplastic kind of flow is that the thickness of the cream decreases when applied to the force, which means the cream undergoes shear thinning. Curved rheograms in pseudoplastic were caused by the shearing work of Long-chain materials such as linear polymers, with increasing shearing stress, ordinarily irregular. Molecules begin to arrange the Long axis in the directional flow. This directive reduces the resistance of the material and results in a higher shear for each subsequent shearing stress (pharmaceutical physics). The molecular arrangement demonstrated that the cream has a natural spread when applied pressure.

The results of this curve indicate that a 10% concentration cream has a non-Newtonian flow, which means that a liquid does not show a linear relationship between changes in force (shearing stress) with its viscosity. The viscosity of the cream decreases when applied, so the cream undergoes shear thinning, and this type of flow is called pseudoplastic.

Pseudoplastic viscosity decreases as the rate of shear increases. Real viscosity is obtained at each rate of shear price from a tangent (tangent line) on the curve at a certain point (typical). But the best description for a pseudoplastic material is a plot of a curve that is overall consistent.

Curved rheograms in pseudoplastic were caused by the shearing work of Long-chain materials such as linear polymers. With increasing shearing stress, normally, the irregular molecules begin to arrange the Long axis in the directional flow. This directive reduces the resistance of the material and results in a higher rate of shear for each subsequent shearing stress (pharmaceutical physics).

The results of this curve show that the 15% concentration cream has a Non-Newtonian flow, which means a liquid does not show a linear relationship between changes in force (shearing stress) and its viscosity. The cream's viscosity decreases when applied, so the gel undergoes shear thinning, and this type of flow is called pseudoplastic. Pseudoplastic viscosity decreases with an increasing rate of shear. Real viscosity is obtained at each scale of sheer price from the tangent (tangent line) on the curve at a certain point (typical). But the best description for a pseudoplastic material is a plot of a curve that is overall consistent. Curved rheograms in pseudoplastic were caused by the shearing work of Long-chain materials such as linear polymers. With increasing shearing stress, typically irregular molecules begin to arrange the Long axis in the directional flow. This directive reduces the resistance of the material and results in a higher rate of shear for each subsequent shearing stress (pharmaceutical physics).

The homogeneity test demonstrated that in the

concentration of 5%, 10%, and 15%, the cream could not be inhomogeneous form leading to a green spot formation.

The organoleptic test demonstrated that the cream with a concentration of 5%, 10%, and 15% has a distinctive form compared to a semi-solid one. The preparation color appears to be the same between 5% and 10% concentration, i.e., which is light green while the 15% concentration looked green. The higher concentration refers to a richer color because of the addition of *C. Vulgaris* extract. The odor in the preparations has a similar fragrance smell in between three concentrations and masked the fishy odor of *Chlorella Vulgaris*.

The test results obtained in the cream concentration of 5%, 10%, and 15% are cream preparations that have been centrifuged for 5 minutes storage does not occur in phase separation so that the resulting cream preparations remain stable and are not affected by gravity.

The irritation score was obtained by averaging the daily irritation test score. A value of 0-0.4 means very mild irritation, 0.5-1.0 mild irritation, 1.1-2.0 moderate irritation, > 2.0 severe irritation. The test results obtained 0, which means the ointment concentration of 5% does not irritate or very mild irritation.

The irritation score is obtained by averaging the daily irritation test score. A value of 0-0.4 means very mild irritation, 0.5-1.0 mild irritation, 1.1-2.0 moderate irritation, > 2.0 severe irritation. The test results obtained 0 results, which means the ointment concentration of 10% does not irritate or very mild irritation.

The irritation score is obtained by averaging the daily irritation test score. A value of 0-0.4 means very mild irritation, 0.5-1.0 mild irritation, 1.1-2.0 moderate irritation, > 2.0 severe irritation. The test results obtained 0 results, which means the ointment concentration of 10% does not irritate or very mild irritation.

## DISCUSSION

This study uses *Chlorella Vulgaris* extract made with a cream preparation with a concentration of 5%, 10%, and 15%, to determine the level of concentration that has the most appropriate formulation and does not cause irritation in test animals.

It can be seen in the spread test that the cream with a concentration of 5% has the highest spreadability compared to the preparation of creams with other concentrations, with a spreading capacity of 149.75 mm<sup>2</sup> or 2,995 cm. The parameters of good cream dispersion are 5-7 cm<sup>10</sup>. The spreadability of the three formulas ranges from 2.0-3.0 cm. The spread test results (Table 3) show that the higher the concentration of *Chlorella Vulgaris* extract in the cream, the smaller the spread power. All cream preparations do not meet requirements. The spread of the cream is less than the standard spread of good topical preparations.

In the pH test, it was found that 5%, 10%, and 15% cream preparations have alkaline properties, this was caused by the type of active ingredient *Chlorella Vulgaris*, which has a high base level that is pH10. The level of acidity or pH is close to neutral, 6-7, so as not to disturb the tissues in the area such as teeth and gums. All preparations are above the normal pH of the mouth. But the cream preparation

with a concentration of 5% has the lowest base level of 8.35, so it has satisfactory results compared to other concentrations.

The viscosity test showed that cream with a concentration of 15% had a viscosity compared to cream preparations with other concentrations, namely the highest, 32.266 Pa.s or 3,226 cps. Based on the data (table 5), the higher the amount of *Chlorella Vulgaris* extract added to the cream, the higher the cream's viscosity value. Requirements for a good viscosity test on shrimps are 4000-40000 cPs, whereas the viscosity of the three concentrations ranges from around 1500 cps - 3500 cps. All cream preparations do not meet the requirements of good viscosity. But cream preparations with a concentration of 15% have a viscosity value close to the standard excellent viscosity requirements.

The results of the type of flow showed that the cream concentration of 5%, 10%, and 15% has a Non-Newtonian flow which means, a liquid does not show a linear relationship between changes in force (shearing stress) with its viscosity. The curve shows the mean curve; the type of flow is pseudoplastic. The pseudoplastic kind of flow is that the viscosity of the cream decreases when applied to the force, which means the cream undergoes shear thinning.

Pseudoplastic viscosity decreases with an increasing rate of shear. Real viscosity is obtained at each rate of shear price from a tangent (tangent line) on the curve at a certain point (typical). But the best description for a pseudoplastic material is a plot of a curve that is overall consistent.

Curved rheograms in pseudoplastic were caused by the shearing work of Long-chain materials such as linear polymers. With increasing shearing stress, normally irregular molecules begin to arrange the Long axis in the directional flow. This directive reduces the resistance of the material and results in a greater rate of shear for each subsequent shearing stress. All our data suggested that the gel has the property of easily spread when applied pressure. Furthermore, all three concentrations of the cream were not homogeneous because they do not meet the requirements of the homogeneity of cream preparations, i.e., the absence of coarse granules.<sup>7</sup>

The organoleptic cream concentration of 5%, 10%, 15%, and type M / A have a semi-solid form and have a distinctive fishy odor of original *Chlorella Vulgaris*. The prepared cream color appears darker with increasing extract concentration, 15% cream has the darkest color compared to the concentration of 5% and 10%. Darker color was due to the more color concentration.<sup>8,9</sup>

Centrifugation test results were conducted to determine the extent of the effect of increasing the concentration of *Chlorella Vulgaris* extract on the physical stability of a cream. With varying concentrations, it can affect the physical stability of a cream. Cream concentrations of 5%, 10%, and 15%, which have been centrifuged for 5 minutes at a speed of 300rpm, phase separation does not occur. Thus, the resulting cream preparations remain stable and are not affected by gravity. Creams with concentrations of 5%, 10% and 15% have good storage stability.<sup>10,11</sup>

The irritation test results, i.e., the score of irritation value obtained by evenly leveling the irritation test score every day. A value of 0-0.4 means very mild irritation, 0.5-1.0

mild irritation, 1.1-2.0 moderate irritation, > 2.0 severe irritation. The test results on the cream concentration of 5%, 10%, and 15% obtained 0 results, which means the 10% concentration cream does not irritate or very mild irritation.<sup>12-14</sup>

## CONCLUSION

The results of formulation and irritation tests performed at the Pharmaceutics Laboratory and Biopharmacy Laboratory of the Faculty of Pharmacy Hasanuddin University concluded that cream with any concentration does not irritate the oral mucosa of the test animal after the application of the oral mucosa is applied to the test animal.

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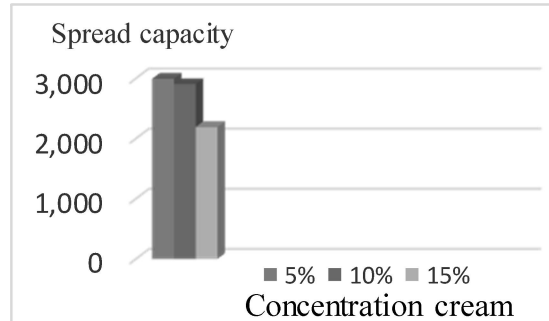
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**Table 1.** Results of spreadability

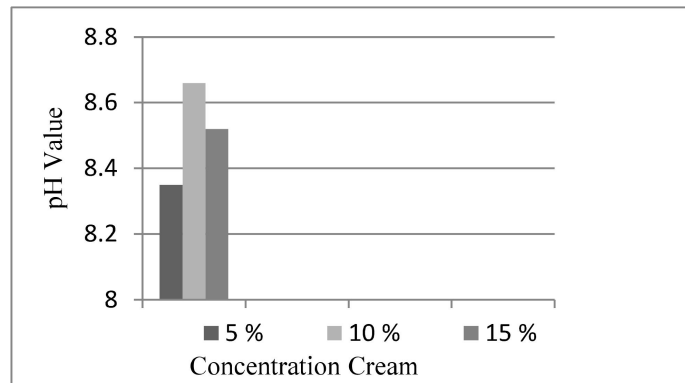
Cream	5%	2,995 cm
	10%	2,906 cm
	15%	2,192 cm



**Graph 1.** Graph of spreadability test

**Table 2.** Results of pH test

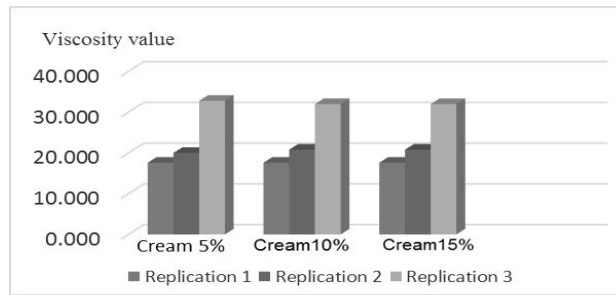
CREAM	5%	8.35
	10%	8.66
	15%	8.52



**Graph 2.** Graph of the results pf pH test

**Table 3.** Result of viscosity test

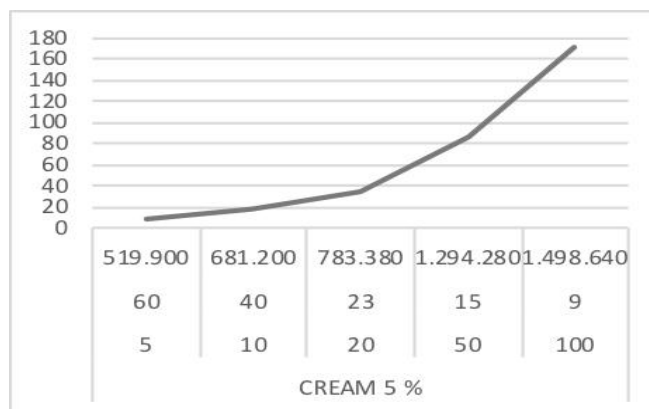
CREAM	5%	17.6
		17.6
		17.6
	10%	20
		20.8
		20.8
	15%	32.8
		32
		32



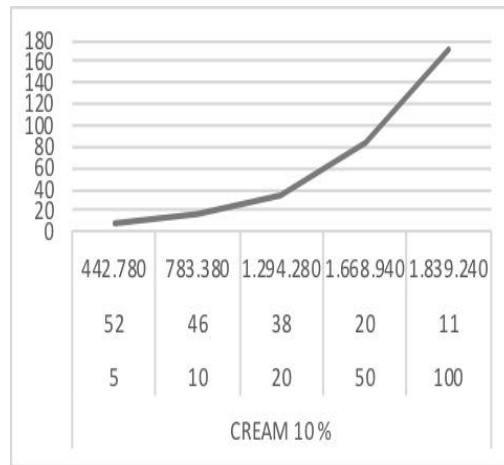
**Graph 3.** Graph of the results of viscosity test

**Table 4.** Rheology test result

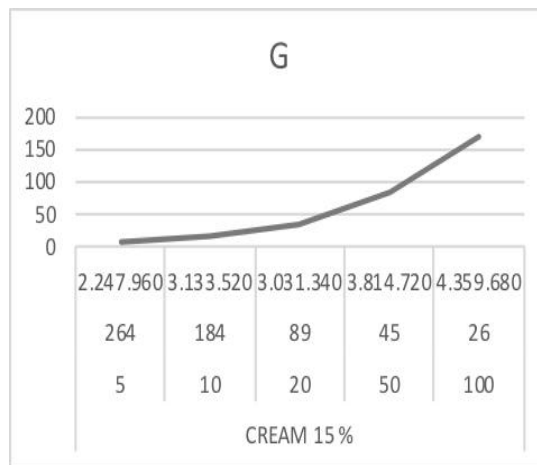
Formula	Rapidity	Viscosity	F	G
Cream 5%	5	60.000	519.900	8.515
	10	40.000	681.200	17.03
	20	23.000	783.380	34.06
	50	15.200	1.294.280	85.15
	100	8.800	1.498.640	170.3
Cream 10%	5	52.000	442.780	8.515
	10	46.000	783.380	17.03
	20	38.000	1.294.280	34.06
	50	19.600	1.668.940	85.15
	100	10.800	1.8399.240	170.3
Cream 15%	5	264.000	2.247.960	8.515
	10	184.000	3.133.520	17.03
	20	89.000	3.031.340	34.06
	50	44.800	3.814.720	85.15
	100	25.600	4.359.680	170.3



**Graph 4.** Graph of rheogram 5% cream concentration



**Graph 5.** Graph of rheogram 10% cream concentration



**Graph 6.** Graph of rheogram test result of 15% cream concentration

**Table 5.** Homogeneity test results

Formula		Homogeneity
L Cream	5%	Inhomogeneous
	10%	Inhomogeneous
	15%	Inhomogeneous

**Table 6.** Homogeneity test result

Formula		Shape	Color	Smell
CREAM	5%	Semi solid	Light green	typical extract
	10%	Semi solid	Light green	typical extract
	15%	Semi solid	Green	typical extract



**Table 7.** Centrifugation test results

Formula		Centrifugation
Cream	5%	No separation occurred
	10%	No separation occurred
	15%	No separation occurred

**Table 8.** Irritation scores of oral mucosa and labial junction

Rating	Oral Mucosa	Labial junction
0	Negative	Negative
1	Discolored, slightly flaking	Redness, flaking, dry mucosa
2	Exfoliate in several areas	Pain, dry and rough mucosa
3	Ulceration	Broke and bleed

**Table 9.** Irritation test results of the concentration of 5%

Test Animal	Weight	Day				
		0	1	2	3	6
1	120 gr	0/0	0/0	0/0	0/0	0/0
2	120 gr	0/0	0/0	0/0	0/0	0/0
3	120 gr	0/0	0/0	0/0	0/0	0/0

**Table 10.** Cream irritation test results 10% concentration

Rating	Oral Mucosa	Labial junction
0	Negative	Negative
1	Discolored, slightly flaking	Redness, flaking, dry mucosa
2	Exfoliate in several areas	Pain, dry and rough mucosa
3	Ulceration	Broke and bleed

**Table 11.** Irritation test results of the concentration of 15%

Test Animal	Weight	Day				
		0	1	2	3	6
1	120 gr	0/0	0/0	0/0	0/0	0/0
2	120 gr	0/0	0/0	0/0	0/0	0/0
3	120 gr	0/0	0/0	0/0	0/0	0/0