

RESEARCH ARTICLE

The effectiveness of *Chlorella vulgaris* cream applied to male and female rats

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ABSTRACT

Background: One of the internal factors of premature aging is free radicals. Free radicals that are produced excessively in the skin will damage the collagen in the skin cell membranes, making the skin less elastic, and causing wrinkles or aging. Chemical compounds that can counteract free radical reactions are antioxidants. *Chlorella vulgaris* contains secondary metabolites such as flavonoids and phenolics which have antioxidant activity. This active component can be used as the main ingredient in safe cream preparations with natural solvents such as avocado seed oil. Avocado seeds contain lots of vitamins and fatty acids so they are safe to use as a natural solvent. **Aims and Objectives:** This study aims to measure the effectiveness of *C. vulgaris* on the skin tested on tested animals. **Materials and Methods:** The materials used in this study were stearic acid, *C. vulgaris*, avocado seed oil, aquadest, niacinamide, pentanol, and other cream production ingredients. The method used was direct testing on female and male rats by rubbing it once a day on the rats' backs for 28 days. Previously, the antioxidant and toxicity values had been carried out using the 2,2-diphenyl-1-picrylhydrazyl and brine shrimp lethality test methods. Furthermore, the measurement of water content, oil content, and skin texture of the test animals was carried out. As a control cream, it was used without the addition of *C. vulgaris*. **Results:** *C. vulgaris* cream has a pH of 5.5 with a viscosity of 15,200 cps. The result of smearing carried out on male and female rats was cream with the addition of *C. vulgaris* which had an increase in water content, higher oil, and a smoother texture than cream without the addition of *C. vulgaris*. The changes were more subtle in the back of the female rats with the proportion of increasing water content for 28 days of 14.6% and oil content of 6.6%. **Conclusions:** *C. vulgaris* cream can be used as a cream that is safe to use with high antioxidant value and changes in skin texture which is better seen from the value of moisture and oil content.

KEY WORDS: *Chlorella vulgaris*; Antioxidant; Rats; Free Radical

INTRODUCTION

The aging process is something that is sure to happen to every human being, but with the development of the times and

human activities that are getting denser, it causes aging to happen even more. Many cases of premature aging occur at a relatively young age of around 20 years. Premature aging is caused by an unhealthy lifestyle, pollution, cigarette smoke, and sunlight, in addition to internal factors from the body that can produce free radicals.^[1]

Free radicals that are produced in excess in the skin will damage the collagen in the skin cell membranes so that the skin lacks elasticity and causes wrinkles or aging, chemical compounds that can counteract these free radical reactions are compounds that contain antioxidants.^[2] Aging process

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shows positive relationship with free radical generation and thereby with oxidative stress.^[3]

Antioxidants can donate one or more electrons to free radicals so that these free radicals can be suppressed and do not damage body cells. Antioxidant compounds can be found in nature, one of which has the active component which is microalgae such as *Chlorella vulgaris*.^[4]

C. vulgaris contains secondary metabolites such as flavonoids and phenolics, which have antioxidant activity.^[5] *C. vulgaris* which has active components such as antioxidants can be used as the main ingredient for safe cream preparations. However, it is not only the ingredients used in the manufacture of anti-aging creams that are dangerous but also the solvents used usually come from organic solvents such as n-hexane which can be toxic and dangerous if applied to the skin.^[6,7]

A natural solvent alternative that is safe to use to extract secondary metabolites is the solvent from avocado seed oil. Avocado seeds contain lots of vitamins and fatty acids so they are safe to use as a natural solvent.^[8,9]

C. vulgaris cream before being applied to human skin, first tested on test animals to see side effects and skin irritation.

MATERIALS AND METHODS

The materials used in this research include *C. vulgaris* powder, avocado seeds, 2,2-diphenyl-1-picrylhydrazyl, 2% NaOCl, shrimp larvae, ether, Veet, alcohol, glycerol, stearate acid, niacinamide, panthenol, filter paper, label paper, and tissue roll. Moreover, the equipment used in this study include tools used in laboratories, centrifuges, digital scales, Erlenmeyer, pipettes, vial bottles, US-110 ultraviolet-visible spectrophotometers, pH meter, viscometer, and skin analyzer.

Production of *C. vulgaris* Cream

The basic formula for type m/a cream is oil in water according to the standards of the Indonesian Pharmaceutical Association. After weighing, the ingredients contained in the formula are separated into two groups, namely, the oil phase (avocado oil and *C. vulgaris* powder, stearic acid, triethanolamine (TEA), niacinamide, and pentanol) and the water phase (aquadest, nipagin, and nipasol).

The oil phase is poured into the hot mortar and the water phase is added gradually while stirring until a creamy mass is formed. Dissolve *C. vulgaris*, avocado seed oil, and stearic acid in hot mortar and added TEA. Then, dissolve nipagin and nipasol with propylene glycol and added aquadest. Pour aqueous phase into the oil phase while stirred in a hot mortar thus forming a cream and added niacinamide and pentanol.^[10]

Microbacterial Testing of *C. vulgaris* Cream

The sample was diluted with sterile diluent to 10^{-6} then homogenized. One milliliter of each sample dilution is pipetted into a sterile Petri dish duplo, then 12–15 mL of liquid plate count agar media are poured. Shake the Petri dish slowly until the samples are well blended. The mixture was allowed to solidify, then put in an incubator ($35 \pm 1^\circ\text{C}$) in an inverted position for 24 h. The number of microbial colonies in 1 g or 1 mL sample was calculated by transferring the colony average number on a plate to the diluent factor used.

The Effectiveness Testing of *C. vulgaris* Cream

The test was carried out randomly on test animals as controls. Examination of skin texture before and after applying the cream. Cream is used by means of shine through the test is cleaned first then the cream is applied to certain parts at night and in the morning regularly. This is done for 28 days to see the effects of the anti-aging cream. Control cream (does not contain *C. vulgaris*) was used as a control cream. Besides that, you can also find out the side effects and reactions on the skin.

Three basic principles of using test animals in general health research ethics are listed in the World Medical Association, namely, respect (respecting the rights and dignity of living things, freedom of choice and desire, and being responsible for themselves, including experimental animals), beneficiary (useful for humans and other creatures, the benefits obtained must be greater than the risks received), and justice (being fair in using experimental animals).^[11,12]

RESULTS

C. vulgaris Cream

The preparation of *C. vulgaris* cream uses avocado seed oil extract and *C. vulgaris* powder with a ratio of 1:20 as the active substance in the cream preparation. The cream from *C. vulgaris* is made with the oil in water (M/A) type. The emulsion that occurs consists of oil droplets dispersed into water, where the oil is internal and water is the external phase. Nipagin and nipasol are used in cream preparations as additives that function as preservatives. Aquadest is used as the main ingredient of cosmetics which functions to provide a cool feeling to the skin and as a skin moisturizer.^[13] TEA is used in this cream preparation as an emulgator because TEA can neutralize fatty acids, adjust pH, and can dissolve oil and water-insoluble materials. The formulations can be seen in Table 1.

Cream of *C. vulgaris* has a pH of 5.5 with a viscosity of 15,200 cps and after a month of storage, the pH becomes 6.5 with a viscosity of 14,400 cps. The pH of *C. vulgaris* cream is in accordance with the pH of human skin so it will not irritate or scaly skin. *Chlorella vulgaris* cream and control cream as shown in Figure 1.

Microbacterial of *C. vulgaris* Cream

Microbial contamination was tested to determine the presence of microbes in *C. vulgaris* cream preparations and the control as a comparison was cream without the addition of *C. vulgaris*. The microorganisms in cosmetics can cause decay or chemical changes in the product and may be harmful by causing an infection of the skin. The result was that *C. vulgaris* cream had a value of 21×10^{-3} colony-forming unit (CFU) and control cream 32×10^{-3} CFU [Figure 2].

The Effectiveness of *C. vulgaris* Cream

The effectiveness of *C. vulgaris* cream was tested on tested animals, namely, female rats and male rats. Rubbing was done once a day on the back of the test animals and observed every day. Before and after basting, the results were measured the humidity and oil content using a skin analyzer [Figure 3].

The increase in moisture and oil content in the test animals smeared with *C. vulgaris* cream was higher than the cream without the addition of *C. vulgaris*. *C. vulgaris* cream applied to female rats increased moisture and oil content by 14.6% and 6.6% while in female mice cream without the addition of *C. vulgaris* added moisture only increased by 3.6% and oil content by 1.6%, as shown in Table 2.

DISCUSSION

C. vulgaris cream applied to female mice increased moisture, oil and texture higher than that applied to male mice. This was predicted because of differences in skin thickness due to different male and female hormones, sebum, and collagen

production.^[14,15] *C. vulgaris* extract can stimulate the synthesis of collagen in the skin and reduce wrinkles.^[16]

Initial toxicity and antioxidant tests and Fourier transform infrared (FTIR) on cream sample preparations, namely, avocado seed oil and *C. vulgaris* have been carried out by Putri *et al.* (2019). The LC₅₀ value of avocado seed oil is 10,000 ppm, which is above 1000 ppm means that it is non-toxic, can be used to extract *Chlorella vulgaris* as a material for production cosmetic. Avocado seed oil and *C. vulgaris* have antioxidant activity 24.78%. It can be seen from the FTIR spectra that *C. vulgaris* contains secondary metabolites such as flavonoids, phenolics, and ascorbic acid which act as antioxidant compounds (Putri *et al.*, 2019). Free radicals produce oxidative stress. Most commonly involved free radicals are superoxide, hydroxyl, alkoxy, peroxy, and nitric oxide (NO). Free radicals have extremely short half-lives ranging from nanoseconds to seconds. The hydroxyl radical is shortest, and NO is longest having half-life of one nanosecond and 1–10 s, respectively.^[17]

The follow-up test to the final stage is the application of cream to the test animals by taking into account the microbacteria,

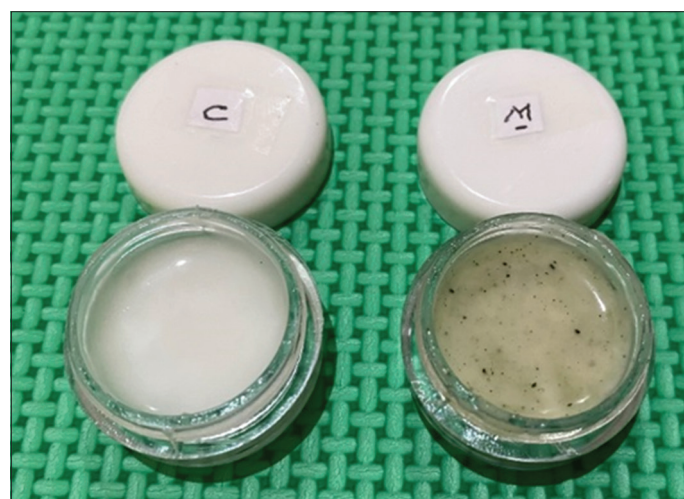


Figure 1: *Chlorella vulgaris* cream

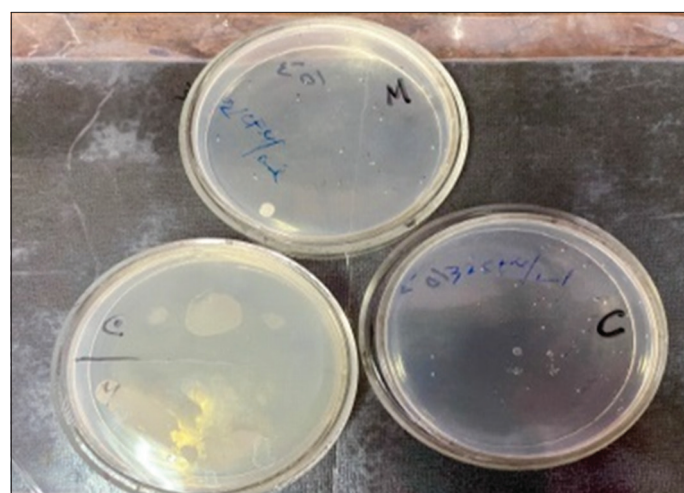


Figure 2: Microbacterial testing of *Chlorella vulgaris* cream

Table 1: Formulation of *C. vulgaris* cream

Materials	Control	<i>Chlorella vulgaris</i>
<i>Chlorella vulgaris</i>	x	20 g
Stearic acid	15 g	15 g
Triethanolamine	1 mL	1 mL
Avocado oil	20 mL	20 mL
Nipagin	0.1 g	0.1 g
Nipasol	0.05	0.05
propylene glycol	5 mL	5 mL
Aquadest	100 mL	100 mL
Niacinamide	2 g	2 g
Pentanol	2 g	2 g

Table 2: Change in skin moisture and oil percentage

Category	Sample			
	MB	CB	MJ	CJ
Water (%)	14.6	3.6	6.1	0.9
Oil (%)	6.6	1.6	2.8	0.5

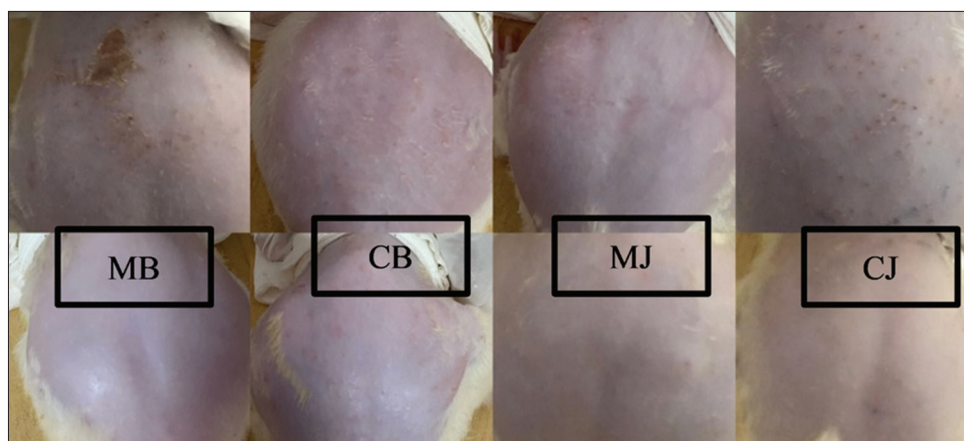


Figure 3: The effectiveness of *Chlorella vulgaris* cream was tested on tested animals. MB: *C. vulgaris* cream in female mice; CB: Cream without *C. vulgaris* in female rats; MJ: *C. vulgaris* cream in male rats; CJ: Cream without *C. vulgaris* in male rats

pH, and viscosity of *C. vulgaris* cream which has been carried out in this study. *C. vulgaris* cream shows good results and safe from the laboratory and clinical tests described above.

CONCLUSIONS

C. vulgaris cream can be applied directly to the skin because it has a pH that matches human skin and is very effective in being applied to female test animals because the active substance in the cream reacts more quickly and enters the skin stratum corneum and does not cause irritation.

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