



Effect of *Chlorella pyrenoidosa* Microalgae Extract and Powder Mixture on Skin Rejuvenation and Antioxidant Enhancement in the Formulation and Evaluation of a Facial Peeling Cream with Natural Ingredients

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ABSTRACT

Currently, the demand for natural cosmetics free of synthetic materials is increasing among women and men in order to have pure, healthy, and attractive skin without potential risks. Therefore, The primary goal of this research is to develop a cream designed for facial exfoliation. with high-quality natural ingredients and with the addition of *Chlorella pyrenoidosa* as a main ingredient in the form of powder and extract at the same time. The ingredients of the cream have peeling, moisturizing, antiseptic, antioxidant, antimicrobial, anti-aging, anti-acne, scar, tanning, and wrinkle treatment properties, as well as nourishing and lightening the skin. In this preparation, algae, activated charcoal, gel prepared from aloe vera, lavender essential oil, coconut essential oil, glycerin, blue indigo, and turmeric are used. The scrub product was evaluated based on physical and chemical criteria, including appearance, texture, smell, PH, density, viscosity, The cream's spreadability, washability, and irritability were evaluated. All the necessary properties were found to be satisfactory. Therefore, the current formulation can be used as an effective exfoliating cream to achieve clean, healthy, and radiant skin.

Keywords: *Chlorella pyrenoidosa* Microalgae, Extract and Powder, Antioxidant Enhancement, Skin Rejuvenation, face peeling cream preparation, Natural Ingredients.

Introduction

The concept of beauty and cosmetics has been deeply embedded in human civilization, with early formulations primarily utilizing natural ingredients derived from plants, minerals, and animals. The widespread use of synthetic ingredients only became prominent in the 20th century. However, the 21st century has witnessed a renewed focus on natural ingredients, driven by consumer demand. This shift has led many cosmetic companies to increasingly replace synthetic components with natural alternatives in their formulations [1]. The cream is easy to spread, easy to wash off, and has a tendency to irritation. All the required properties have been shown to be good. Therefore, we can use the present formulation to protect the skin from diseases and as an effective peeling cream to obtain clean, healthy and radiant skin. Cosmetics and skin care products may contain ingredients whose safety is not confirmed or are known to pose health risks [2]. Most cosmetics available in the market and shops contain dangerous chemicals such as sodium laureth sulfate, parabens, phthalates, triethanolamine, and some heavy metals such as lead, arsenic, nickel, cadmium, and mercury. Bioaccumulation of these harmful chemicals and metals in the body over time has been linked to cancer, Reproductive disorders, dermatitis and allergies can occur when chemicals reach the body through the skin through absorption and penetration of harmful chemicals from creams into the body [3][4][5].

In recent years, some research has emerged in the field of skin care products that use natural materials instead of chemicals. Interest has begun in using algae in these products due to their great health benefits. Some researchers have used algae in powder form, for example, in a study by Firdayanti Paputungan et al., 2023. This study reveals that incorporating *Eucheuma spinosum* algae powder into exfoliating bath soap significantly enhances its antioxidant activity. Soaps produced through cold and hot processes showed DPPH inhibition rates of 76.76% and 70.13%, respectively, compared to soaps without algae powder [6].

In other studies, using microalgae extract as a main ingredient to prepare skin cleansing soap was found to provide protection from the effect of free radicals. *Chlorella pyrenoidosa* contains compounds with antioxidant properties that can inhibit free radicals [7].

Studies are still ongoing and research is underway to obtain impressive results. In this study, to address the issue of dead cells accumulating on the face and protect it from free radicals, wrinkles, bacteria that cause acne, and the damage of chemicals.

To enhance the effect of the peeling cream with natural ingredients, we used microalgae powder and its extract at the same time and in equal proportions in the components of the exfoliating cream with activated charcoal prepared from date pits and white clay (kaolin clay) and some natural materials. It has also been proven that microalgae in cosmetics and skin care products have the ability to protect from the sun, care for hair, provide moisturization, revitalize or rejuvenate care products, act as anti-aging creams, and function as anti-irritant peels [8][9][10][11].

MATERIALS AND METHODS

The measuring devices including pH meter, viscometer, centrifuge, drying oven, freeze dryer, and electric mixer were used. Microalgae (*Chlorella pyrenoidosa*), distilled water, and activated carbon were prepared from date pits. Algae powder preparation, algae extraction, and oils used in the formulation were performed at the Research Center of Ouargla University, Algeria. Local white clay was obtained from a clay quarry. The materials for the formulation **Table 1**. Of the cream were purchased from an export and import company specializing in these materials in Algeria. They were verified and stored.

Microalgae preparation

Chlorella pyrenoidosa strain was used in this study. All inoculations were performed under sterile conditions. 6 mL (7.5×10^6 cell/mL) of *C. pyrenoidosa* was inoculated in 500 mL of Autoclaved BG11 medium [12].

Once a sufficient density was reached, they were inoculated in 15 L glassware. All cultures were exposed to an irradiance of 27 $\mu\text{mole photons m}^{-2} \text{ s}^{-1}$. Mixing was achieved by continuous bubbling with air. The temperature was maintained between 24 and 26 °C, pH 7-8 under aseptic conditions [13].

Preparation of *C. pyrenoidosa* powder

After the algae have grown to a suitable stage, the algae cells are separated from the water by filtration or centrifugation. The sediment (algae paste) is collected, and the water is discarded. The dry biomass of the microalgae *Chlorella pyrenoidosa* is obtained by drying the algae biomass in an oven at a temperature below 38°C for a minimum of 24 hours to preserve the bioactive compounds. After the biomass is thoroughly dried, it is ground into a fine powder. The algae powder is packed in airtight containers and stored in a cool, dry place to maintain its quality and nutritional value [14].

Preparation of *C. pyrenoidosa* extract

To prepare the extract, the Soxhlet extraction method was used. 2 g of freeze-dried biomass powder was weighed. The extraction process takes between six and twenty hours at a temperature of 140°C. We used a solvent of 52 mL of pure n-hexane alone or as a mixture (39 mL hexane + 13 mL ethanol). Followed by rinsing the solvent for 30 min and evaporating the solvent for 30 min [15].

Preparation of Activated Charcoal from Date Pits

Activated charcoal is an excellent absorbent material due to its highly porous structure and large surface area; it traps fine particles, dead cells, dirt, bacteria, toxins, and chemicals on the skin surface, helping you achieve flawless skin.

We prepare activated charcoal from the pits of Algerian dates, called Deglet Nour, which are a low-cost material. This is done by washing them well with distilled water to remove any residual flesh and impurities. And then drying them in the shade under the sun until completely dry. Carbonize the pits at (500-900°C) in an inert (e.g., under nitrogen gas) for 2-4 hours. The

inert gas prevents combustion and helps in developing the porous structure. We impregnate the charred date kernels with the activation agent (KOH or ZnCl_2) at a concentration of 1:1 by weight by soaking them in the solution for 24 hours. We remove the excess solution and dry the impregnated material. We heat the impregnated date kernels in the oven at a lower temperature (usually 400-700 °C) than the roasting temperature for 1-2 hours in an inert atmosphere. Physical activation can be done by exposing the charred material to steam or carbon dioxide at high temperatures (800-1000 °C). After activation, we cool the material to room temperature, then we grind the activated charcoal, we screen the ground material to obtain uniform particle size, washing and drying, we wash the activated charcoal with distilled water to remove any residual chemicals, we dry the washed activated charcoal in an oven at 105°C until completely dry [16][17].

Preparing the Exfoliating Cream

There are two stages in preparation:

Preparation of the oil phase:

Appropriate amounts of oil phase materials such as glycerin, beeswax, coconut essential oil, lavender essential oil, and lanette emulsifier are placed in a beaker.

Preparing the aqueous phase:

Appropriate amounts of aqueous phase materials such as distilled water, blue indigo, zinc oxide citric acid, potassium sorbate, aloe vera, glycerin, , and xanthan gum, are mixed in a beaker. Simultaneously heat the contents of both cups in hot water, ensuring the temperature does not exceed 70°C, for 20 minutes. After removing them from the water bath, the two phases are cooled to approximately 36 °C. Before the oil layer solidifies, we pour the two phases (aqueous phase and oil phase) into one container Add 60% of the prepared aqueous phase in batches to 40% of the oil phase with continuous mixing. Then, incorporate the desired fragrance, color, and appropriate vitamins. The composition of the peeling cream is detailed in **Table 2. [18]**. We obtain a moisturizing exfoliating cream with a creamy texture. Apply the exfoliating cream topically on the face for 15-20 minutes. Rub the face for 2-3 minutes, then wash with fresh, lukewarm water [19].

Composition of Algae Peeling Cream

Table 1: Composition of Algae Peeling Cream

N°	Common Name	Category	Quantity (%)
1	<i>Chlorella pyrenoidosa</i>	Antioxidants ,Skin tightening, Treats facial acne and pimples, Stimulating the growth of new cells and tissues	3+3 Powder and Extract
2	Activated charcoal	Natural exfoliant. Removes dead skin cells.	3

	from the pits of Algerian dates, called Deglet Nour	Charcoal helps refresh the skin by removing all impurities from the skin and revealing radiant and beautiful skin.	
3	white clay (kaolin clay) $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$	It helps absorb deposits and excess oily secretions from the skin, Used as a gentle exfoliator. It helps improve skin texture, tone and tighten skin.	2.5
4	Blue indigo $\text{C}_{16}\text{H}_{10}\text{N}_2\text{O}_2$	Anti-aging, Anti-wrinkle, Skin lightening and whitening	1
5	Aloe vera gel	Antibacterial, Renewing skin cells, tightening sagging skin, reducing the appearance of acne and scars	3
6	Turmeric $\text{C}_{21}\text{H}_{20}\text{O}_6$	Antioxidants, Anti-inflammatory and anti-bacterial. Anti-aging Antiseptic and improves. It is effective in treatment of acne due to its antiseptic and antibacterial properties that fight pimples and breakouts to provide a youthful glow to your skin. Fairness. Vitamin C, E, D.	0.5 [20]
7	Lavender essential Oil $\text{C}_{10}\text{H}_{18}\text{O}$	Antioxidants, Antibacterial, Promotes healing of wounds, bruises and burns	12.5
8	Coconut essential Oil $\text{C}_{12}\text{H}_{24}\text{O}_2$	Resistant to wrinkles and signs of aging. Natural skin exfoliant Soothe the symptoms of psoriasis and eczema	12.5
9	Beeswax	Moisturizer for the skin Natural exfoliant, Suitable for sensitive skin	15
10	Lanette O $\text{C}_{16}\text{H}_{34}\text{O}$	- Emulsifier, thickener and stabilizer. - It increases the thickness of the final product more effectively, it improves beeswax as an emulsifier - It is hydrophilic and is best suited for regulating the viscosity of cosmetic oil/water emulsions.	2
11	Potassium sorbate $\text{C}_6\text{H}_7\text{KO}_2$	An effective preservative active against mold, fermentation and bacteria Typically extends the shelf life of personal care products by several months	.05
12	Xanthan gum $\text{C}_{35}\text{H}_{49}\text{O}_{29}$	It soothes the skin and is used as a thickening agent, forming a gel-like consistency, which greatly helps in bringing the ideal texture and viscosity to skin care products.	1
13	Glycerin $\text{C}_3\text{H}_8\text{O}_3$	Antimicrobial. Moisturizer for the skin. Reduces the appearance of wrinkles and fine lines, Slowing the appearance of signs of aging.	4 [23]
14	Zinc oxide ZnO	Antibacterial, Anti-irritant, Preservative	0.5
15	Distilled water H_2O	Aqueous phase carrier. Maintain optimal skin moisture Prevent dry and cracked skin	36

Table 2: Evaluation of algae facial scrub.

PARAMETERS		OBSERVATION
Organoleptic	colour	Prussian blue
	Odor	Pleasant
	Texture	Fine
	Consistency	Good
Physicochemical	Thermodynamic Stability of pH	6.8 at 4°C 6.9 at 25°C 6.9at 40°C
	Moisture content	6%
	Physical stability	60% aqueous phase and 40% oil phase
General Characteristics	Viscosity	0.14470 Pa.S
	Volumetric mass	1.21g/cm ³
	Ia : Acid index	0.42
	a :Acidity	42%
	Irritability	Applied on face and did not irritate
	Washability	Easily washable
	Nature of Face after wash	Soft and fresh, clean from dirt

***Mean of triplicate readings.**

Results and Discussion

Through **Table 2** - Evaluation of different standards for facial scrub with a *Chlorella pyrenoidosa* it can be said that the prepared peeling cream has successfully passed all evaluation criteria.

The peeling cream was assessed for its fragrance and color, revealing a dark blue color due to the color of indigo and a distinctive smell due to the smell of essential oils used in the formula. Any desired scent can be added to it.

Texture

Visual and tactile feedback indicated that the prepared exfoliating cream had a creamy texture.

PH measurement

The pH of the exfoliating cream was measured at three different temperatures using an electronic pH meter. The results were as follows:

PH = 6.9 at 25°C and 40°C, and PH = 6.8 at 4°C.

This indicates that the exfoliating cream has a physical and chemical stability with respect to temperature because the pH did not change significantly, As noted in the study by Deborah et al. (2020) [21]. And Wayan Sri Agustini et al. [22].

Spreadability

The spreadability of test samples was determined using the following technique: 0.25 g test

formulation was placed within a circle of 1 cm diameter pre-marked on a glass plate over which a second glass plate was placed. A weight of 250 g was allowed to rest on the upper glass plate for 5 min. Spreadability refers to the area covered by a fixed amount of cream sample after the uniform spread of sample on the glass slide. The increase in the diameter due to spreading of the test formulation was noted [23]. Average of three determinations was noted.

Viscosity

The viscosity of the cream was evaluated using a viscometer. A 50 g sample was placed in a beaker and allowed to equilibrate for 5 minutes. and its value was 0.14470 Pa.S. This is an acceptable value for creams in general and this result was very close to the value found by Sachin Bhagwat Aglawe et al 2011 [20].

Irritation

0.5 g of the cream was applied to the face and left for 5 minutes, no irritation was seen. We did this process for seven days with three people with different skin types.

Washability

To determine how easily the cream can be removed with water alone, the cream is applied to the face and rinsed off. It is observed that it can be removed easily.

Physical stability study:

We prepared nine samples with different ratios of aqueous phase and oil phase without adding emulsifiers in test tubes (10% oil phase and 90% aqueous phase, until we reach 90% oil phase and 10% aqueous phase). We shake the contents of each tube until they are mixed and leave the mixture for a sufficient period of time and record which content is more stable, meaning we wait for the last tube in which the oil phase separates from the aqueous phase. We noticed that the last tube in which the separation occurs was 60% aqueous phase and 40% oil phase, meaning that the prepared formula will be in these proportions so that the oil phase does not separate from the aqueous phase for the longest possible time and the cream can withstand relatively high temperatures.

Acid index

The acid number was calculated by the reflux reaction of the cream in a mixture of ether solvent and alcohol, 25 ml of ethanol and 25 ml of ether solvent are mixed and 4 g of cream is added. The mixture is decondensed until the sample dissolves, in order to calibrate the sample solution, a 0.1 mol/L solution of potassium hydroxide is prepared and the sample solution is calibrated. We add a few drops of phenolphthalein, the solution is calibrated and a light purple color appears, then the volume of potassium hydroxide used to calibrate the cream solution is read, then we calculate the value from the relationship:

$$I_a = \frac{c.v.M}{m} \quad (1)$$

Ia: Acid index

C: Concentration of potassium hydroxide.

The volume of potassium hydroxide poured in the titration process at the equivalence point is 3 cm³, Ia =(0.1×3×56)/4 , (Ia =4.2)

A pH of 4.2 within the range (from 2 to 8) means that the product will have a significant shelf life

Acidity:

The acidity is calculated from the results obtained in calculating the acid index , by the titration method.

$$a = \frac{v.c.M}{10.m} \quad (2)$$

$$a = \frac{I_a}{10} \quad (3)$$

Ia: Acid index.

a: Acidity.

V: Average volume in ml of potassium hydroxide solution used in the titration.

C: Concentration in moles/liter of the titrated potassium hydroxide solution.

M: Molar mass, in grams/mole.

m: Mass, in grams, of the cream.

The acidity was calculated and we got the following result: a= (0.1×3×56)/10×4, a = 42%.

Characterization by IR spectrophotometry

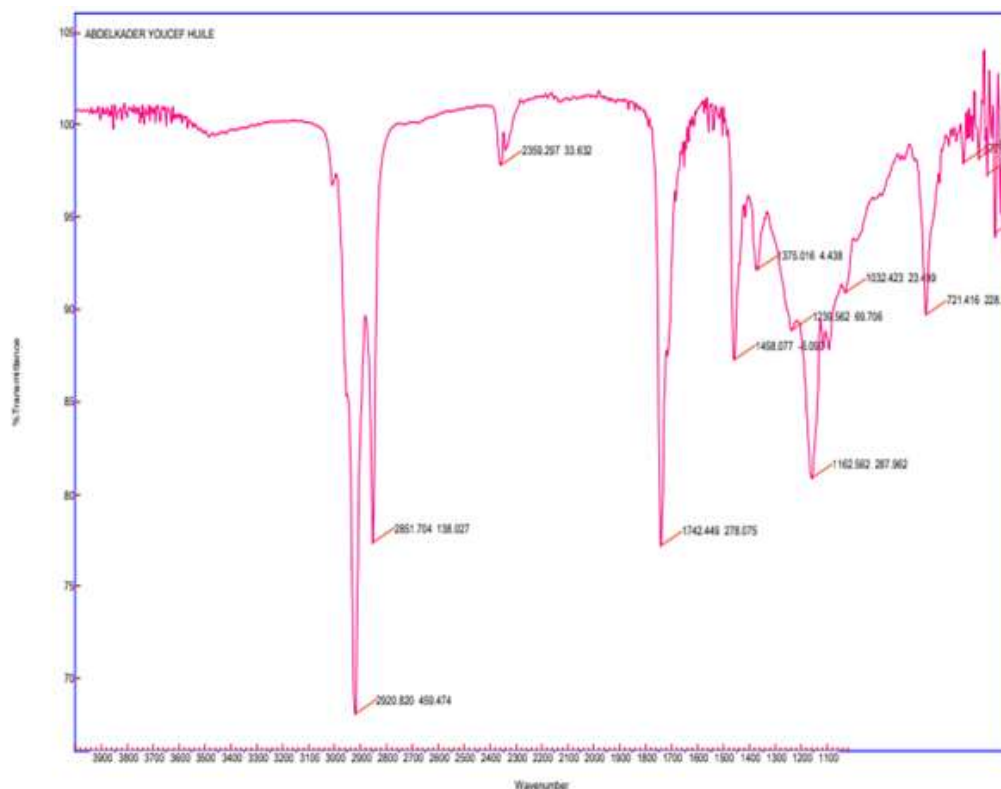


Figure 1. Characterization by IR spectrophotometry

Interpretation of infrared spectrophotometry

In Figure 1, we notice the most important main absorption bands such as:

2920.82 $1/\text{cm}$ representing the asymmetric methylene group CH_2 (lipid), 2851.7 ($1/\text{cm}$) CH_2 symmetric CH_2 (fat) and CH_3 is methylene and the methyl group 1742.4 ($1/\text{cm}$) $\nu \text{C} = \text{O}$ triglyceride, 1461/1458 ($1/\text{cm}$) $\delta_{\text{as}}\text{CH}_3$ and $\delta_{\text{as}}\text{CH}_2$ from lipids, 1239.5 ($1/\text{cm}$) between (1310-1240) Amide III proteins.

This scrub mainly contains microalgae that contain many nutrients that are beneficial to the skin, such as vitamins E, C, K, A, magnesium, calcium, copper, potassium, iron, zinc, dietary fiber, selenium. And all the essential acids and omega-3 fatty acids, etc. Algae are an important element in skin tissues and nutritional supplements because they are beneficial for health and beauty. [24] [25].

Vitamin E, an antioxidant nutrient, helps repair DNA. And copper, which helps in the production of melanin, which gives the skin its pigment [26] [27].

Antibacterial activity test

In this study, the sensitivity of the isolated bacterial species was evaluated using the disk diffusion method (Kirby-Power), as described by Saif et al. (2017).[28][29].

The antibacterial effect of the face peeling cream was tested by selecting four different bacterial strains known for their frequent use and resistance. In the tests conducted on the staphylococcus

strain, the latter presented a picture of its great effect on both the peeling cream and the oils used, and the effect of the salmonella and pseudomonas aeruginosa strains also with the oils and a slight effect on the cream. As for the Escherichia coli strain, there is no effect, as it was also noted that aloe vera responds to the effect of the salmonella and staphylococcus strains.

Table 3. Zones of inhibition against different bacteria

	Strains	Zones of inhibition	Sensitivity
Prepared peeling cream	Escherichia coli	12mm	sensitive (+)
	Pseudomonas aeruginosa	10mm	sensitive (+)
Aloe Vera	Pseudomonas aeruginosa	16mm	Very sensitive (++)
	Staphylococcus aureus	14mm	Susceptible (+)
Coconut Essential Oil Lavender Essential Oil	Salmonella Very	15mm	sensitive (++)
	Pseudomonas aeruginosa	13mm	Sensitive (+)
	Staphylococcus Very	9mm	sensitive (+)

Conclusion

This study aimed to develop a facial peeling cream using multiple natural ingredients to cleanse the face from dead cells, dirt and bacteria, treat wrinkles and protect against acne. The formulation included chlorella microalgae extract and powder, activated charcoal from local date pits (Deglet Nour), local white clay and other natural ingredients. The prepared peeling cream was evaluated using different criteria and found to be effective on the skin, promote health and give a fresh look to the face without side effects. Microbial analysis indicated inhibitory activity. The research suggests that this formulation can provide treatments for wrinkles and protect against acne because the cream has the ability to kill all acne-causing bacteria using herbs, and helps in developing safe and effective herbal formulations for disease management.

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