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THEME

Developing an antioxidant and anti-aging face serum containing spirulina extract, and evaluating its physical, chemical, and biological properties.

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DEDICATION

Praise be to Allah for the successful completion and the best ending.

I dedicate my graduation and the harvest of what I have worked for first to myself, who remained patient and persevered until reaching this moment.

To the one who was the first supporter of my ambitions, the one who was my refuge and my right hand during this stage,
the one through whom I found my way in life, the tender heart whose prayers always surrounded me — my beloved mother.

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This graduation is not just a certificate,
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DEDICATION

I am committed to it, and even if difficulties stand in my way, I have achieved this by the grace and help of God.

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Ikhlas

Abstract

This study falls within the scope of enhancing natural resources and their use in the cosmetic industry. The main objective of this work is to prepare a natural serum based on spirulina *(Arthrospira platensis*) extract and to evaluate its topical efficacy on the skin. For this purpose, the extract was obtained through several stages (defatting, protein extraction, and processing to obtain bioactive compounds), and two serum formulations were developed (one for dry skin and the other for oily skin). These formulations were subjected to physicochemical tests (pH measurement and stability testing). Regarding the in vivo study, 50 µL of each formulation were topically applied to 24 Wistar rats, divided into 6 groups (4 rats per group), in order to assess the serum's effects on the skin through hematological, biochemical, bacteriological, and histological analyses.

Physicochemical analyses revealed that the prepared serum exhibits suitable properties for topical application, with good stability and remarkable skin compatibility. Hematological parameters (Table 05) for the treated group (G2/g4) showed the following values: white blood cells ($5.4 \times 10^3/\mu L$) within the reference range, red blood cells ($6.56 \times 10^6/\mu L$) slightly above the reference, hemoglobin (13.2 g/dL), platelets ($734 \times 10^3/\mu L$) elevated compared to the norm, and hematocrit (41.2 %) within normal limits. Furthermore, biochemical parameters (Table 06) recorded blood glucose levels ranging between 0.58 and 0.76 g/L, urea between 0.30 and 0.37 g/L, and creatinine between 5.90 and 7.10 mg/L, with a low coefficient of determination ($R^2 = 0.08$) for creatinine, indicating low variability among groups.

The in vivo results demonstrated a significant improvement in biological and tissue indicators compared to the control group, with a marked reduction in signs of dryness and inflammation at the skin level. Histological examination confirmed the good tolerance and beneficial effect of the formulations on the structure of the epidermal tissue.

In conclusion, this study confirms the promising potential of spirulina as a viable natural ingredient in cosmetic and dermatological formulations. The demonstrated stabilizing properties, safety profile, and biological efficacy open new perspectives for the application of this cyanobacterium in skincare products.

Keywords: Spirulina, natural serum, skin, biochemical analyses, histological study, cosmetics

الملخص

تندرج هذه الدراسة في إطار تثمين الموارد الطبيعية واستعمالها في الصناعات التجميلية، حيث تمثل الهدف الرئيسي لهذا العمل في تحضير سيروم طبيعي اعتماداً على مستخلص السيرولينا (سبيرولينا) وتقييم فعالية الموضعية على البشرة. لهذا الغرض، تم الحصول على المستخلص عبر مراحل متتالية (إزالة الديون، استخلاص البروتينات، ومعالجتها للحصول على مركبات فعالة)، ثم تطوير تركيبتين من السيروم (للبشرة الجافة والأخرى للدهنية). أما فيما يخص الدراسة في الجسم الحي، فقد تم تطبيق 50 ميكرولتر من كل تركيبة موضعياً على 24 فأراً من الذكور من نوعWistar ، وزعت إلى 6 مجموعات (4 فئران في كل مجموعة)، حيث خضعت العينات لاختبارات فيزيائية وكيميائية (قياس درجة الحموضة واختبار الثبات)، بالإضافة إلى تقييم المعايير الدموية، البيوكيميائية، البكتيرية، والدراسة النسيجية للجلد.

أظهرت التحاليل الفيزيوكيميائية أن السيروم المحضر يتّسم بخصائص مناسبة للاستعمال الموضعي، مع استقرار جيد وتوافق ملحوظ مع البشرة. كشفت القياسات الدموية للمجموعة المدروسة (G2/g4) عن القيم التالية: كريات الدم البيضاء (WBC: 5.4 ×10³/µL) ضمن المدى المرجعي، كريات الدم الحمراء (RBC: 6.56 ×10⁶/µL) أعلى من المرجع، الهيموغلوبين(Hb: 13.2 g/dL) ، الصفيحات (Plt: 734 ×10³/µL) مرتفعة، والهيماتوكريت (Hct: 41.2%) ضمن الحدود الطبيعية. كما بينت المعايير البيوكيميائية (الجدول 06) قيماً تراوحت فيها نسبة السكر في الدم بين 0.58–0.76 g/L، اليوريا بين 0.30–0.37 g/L ، والكرياتينين بين 5.90–7.10 mg/L ، مع معامل تحديد (R^2) منخفض للكرياتينين (0.08) يشير إلى تباين محدود بين المجموعات.

أما على صعيد النتائج في الجسم الحي، فقد أظهرت الدراسة النسيجية تحسناً ملحوظاً في بنية الجلد وانخفاضاً واضحاً في مظاهر الجفاف والالتهاب لدى المجموعات المعالجة مقارنة بالمجموعة الشاهدة. كما أكدت التحاليل البيولوجية تحسناً في بعض المؤشرات النسيجية، مما يعكس الفعالية الموضعية للتركيبتين وسلامة استعمالهما.

في الختام، أثبتت هذه الدراسة النشاط البيولوجي الواعد للسيروم المحضر من السيرولينا، وقدرته على تحسين خصائص البشرة وتخفيف الاضطرابات الجلدية. تجدر الإشارة إلى الإمكانات العلاجية القوية لهذا المكون الطبيعي في مجال مستحضرات التجميل والعناية بالبشرة، مما يفتح آفاقاً جديدة لتطبيقاته في الصناعات الدوائية والتجميلية.

الكلمات المفتاحية: السيرولينا (سبيرولينا)، سيروم طبيعي، البشرة، التحاليل البيوكيميائية، الدراسة النسيجية، مستحضرات التجميل.

Résumé

Cette étude s'inscrit dans le cadre de la valorisation des ressources naturelles et de leur utilisation dans les industries cosmétiques. L'objectif principal de ce travail est de préparer un sérum naturel à base d'extrait de spiruline (*Arthrospira platensis*) et d'évaluer son efficacité topique sur la peau. Pour ce faire, l'extrait a été obtenu par différentes étapes (dégraissage, extraction des protéines et traitement pour l'obtention de composés bioactifs), puis deux formulations de sérum ont été développées (l'une pour peau sèche et l'autre pour peau grasse). Ces formulations ont été soumises à des tests physico-chimiques (mesure du pH et test de stabilité). Concernant l'étude in vivo, 50 µL de chaque formulation ont été appliqués par voie topique sur 24 rats Wistar, répartis-en 6 groupes (4 rats par groupe), afin d'évaluer les effets du sérum sur la peau à travers des analyses hématologiques, biochimiques, bactériologiques et histologiques.

Les analyses physico-chimiques ont montré que le sérum préparé possède des propriétés adaptées à une utilisation topique, avec une bonne stabilité et une compatibilité cutanée remarquable. Les paramètres hématologiques (Tableau 05) du groupe traité (G2/g4) ont révélé les valeurs suivantes : globules blancs ($5,4 \times 10^3$/µL) dans la plage de référence, globules rouges ($6,56 \times 10^6$/µL) légèrement au-dessus de la référence, hémoglobine (13,2 g/dL), plaquettes (734×10^3/µL) élevées par rapport à la norme, et hématocrite (41,2 %) dans les limites normales. Par ailleurs, les paramètres biochimiques (Tableau 06) ont enregistré des valeurs de glycémie comprises entre 0,58 et 0,76 g/L, d'urée entre 0,30 et 0,37 g/L, et de créatinine entre 5,90 et 7,10 mg/L, avec un coefficient de détermination (R^2) faible pour la créatinine (0,08), indiquant une faible variabilité entre les groupes.

Les résultats de l'étude in vivo ont démontré une amélioration significative des indices biologiques et tissulaires par rapport au groupe témoin, avec une réduction marquée des signes de sécheresse et d'inflammation au niveau cutané. L'examen histologique a confirmé la bonne tolérance et l'effet bénéfique des formulations sur la structure du tissu épidermique.

En conclusion, cette étude confirme le potentiel prometteur de la spiruline en tant qu'ingrédient naturel viable dans les formulations cosmétiques et dermatologiques. Les propriétés stabilisatrices, la sécurité d'emploi et l'efficacité biologique démontrées ouvrent de nouvelles perspectives pour l'application de cette cyanobactérie dans les soins de la peau.

Mots-clés : Spiruline, sérum naturel, peau, analyses biochimiques, étude histologique, cosmétiques.

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GA	Aerobic mesophilic germs
EC	Escherichia coli
LM	Yeasts and molds
ST	Staphylococcus aureus
Pseud	Pseudomonas aeruginosa
TSE	Tryptone Salt Water
PCA	Plate Count Agar
BCP	Bromocresol Purple Agar
SAB	Sabouraud Agar
SLS	sodium lauryl sulfate

summary

Introduction

Introduction

In recent decades, the cosmetics industry has witnessed a significant decline in its popularity and credibility among a growing segment of consumers, and this decline has not only come as a result of the change in aesthetic tastes towards a natural appearance, but also due to the growing awareness of the unethical practices that have long characterized this industry, most notably: excessive reliance on harmful chemicals such as parabens, phthalates, and silicones, weak warnings about the health risks associated with them, and the continuation of animal experimentation despite global campaigns against them, as well as the environmental footprint catastrophic caused by millions of non-recyclable plastic packaging and micro-pollutants that pollute water and soil. As global attention shifts towards the values of sustainability, public health, and ethical consumption, it is imperative to understand the industry's profound environmental and health impacts, and to reorient them towards safer and more respectful alternatives for the planet and humans, highlighting the urgent need for natural, bioactive ingredients whose benefits are not only "free of harmful substances", but are effective, scientifically proven, and capable of meeting multiple skins needs**(Ragusa,I,2021**)

The idea of using nature in body care is not a modern invention, but its roots extend back to the earliest human civilizations that relied almost entirely on natural resources, as the ancient Egyptians used essential oils, herbs, and clay, the Chinese and Indians developed complex recipes based on medicinal herbs, while Mediterranean civilizations knew natural mixtures such as honey, olives, and aloe vera. With the development of pharmaceutical chemistry in the twentieth century, these natural preparations declined to be replaced by cheap synthetic formulations, but recent decades have seen a strong return to nature, not in a naïve way that reproduces old recipes, but with a scientific orientation that looks for organisms capable of producing complex molecules that the laboratory could not efficiently imitate, hence the attention has turned to new biological sources such as microalgae and cyanobacteria. Microalgae and cyanobacteria are highly adaptable to harsh environmental conditions, producing unique defensive and bioactive compounds, rich in antioxidants, amino acids, vitamins and natural dyes. Among these organisms, blue-green algae, scientifically known as Arthrosporic and commercially known as spirulina, stands out as one of the most studied and applied species in the cosmetics industry, as spirulina contains a unique synergistic group of active peptides that stimulate collagen production, polyunsaturated fatty acids (especially gamma-linolenic acid) that repair the skin's lipid barrier, phycocyanins as a powerful antioxidant, as well as vitamins (B12, E, beta-carotene) and minerals (zinc, selenium, magnesium), providing a complete healthy function. And integrated for cosmetic formula. Despite these promising possibilities, applied research on the incorporation of spirulina into ready-made and stable cosmetics (such as serums and creams) is still relatively limited compared to other natural substances, as most of the available studies focus on the theoretical aspects or the

use of raw extracts without going through the formulation and standard testing stages of the final product, and most of the products available on the market either use spirulina superficially as a filler, or focus only on its nutritional benefits without investing in its multiple cosmetic properties. Hence, the central problem of this thesis begins**: Is it possible to develop a natural and stable cosmetic serum, based on local spirulina extracts, that combines antioxidant, regenerative and therapeutic efficacy, and meets the requirements of the modern market in terms of safety, stability, and acceptable sensory properties?(2024، عبير عبد الله)**

This study is important in its attempt to bridge the gap between theoretical knowledge of the benefits of spirulina and the practical application of a final cosmetic product, especially in the context of the global trend towards "green beauty" and "clean" products that reject harmful chemicals and animal experimentation and rely on sustainable ingredients. Therefore, this work aims to: scientifically shed light on the documented cosmetic benefits of spirulina by demonstrating its chemical composition and proposed mechanisms of action, developing an innovative cosmetic product (natural serum) containing active spirulina extracts through a precise blend of available natural ingredients, evaluating this product through tests of physical and chemical stability and sensory properties as well as tests of antioxidant efficacy and primary cosmetic and therapeutic effects, and finally presenting an applied model that justifies and enhances the extensive therapeutic and cosmetic use of this algae. Its integration into the beauty and dermatology industry.

To achieve the aforementioned objectives, this thesis has been divided into two main integrated parts: the first part includes the theoretical framework, with an overview of spirulina (its classification, chemical composition, nutritional value) followed by an overview of human skin (its structure, functions, problems) and skin care products (its classification, quality standards, and natural formulation trends). The second part is the applied study, which includes the preparation of the active extract of spirulina using environmentally friendly extraction methods, then the production of the natural cosmetic serum, the study of its stability and sensory properties, and the evaluation of its therapeutic, cosmetic and antioxidant effects, to then analyze and discuss the results in the light of the previous literature, and to come up with final conclusions that summarize the success of the study in achieving its goals, and provide recommendations for future applications and expanded clinical studies.

FIRST PART

BIBLIOGRAPHIC SYNTHESIS

CHAPTER I :

General information about Spirulina

I.1. *Arthrospira plantensis*(spirulina)

Spirulina is one of the most popular dietary supplements in the world. It is a type of blue-green algae and is considered one of the most nutrient-dense foods on Earth ; it contains antioxidants, proteins, and essential amino acids, as well as iron, calcium, potassium, beta-carotene, and many other nutrients.

Thanks to these beneficial components, studies confirm the health benefits of spirulina in several ways; it may aid in weight loss, regulate cholesterol, and promote healthy skin and eyes**. (Lama at all,2024)**

I.1.1 Sources of Spirulina

Spirulina can be obtained from the following sources.

Nature : It grows naturally in freshwater or saltwater lakes

Spirulina farms **:** Spirulina is cultivated in man-made ponds, and spirulina supplements are typically prepared from this type**(lama et al ,2024)**

I.2. Morphological form

Shape : Spiral filaments composed of prokaryotic cells.

Structure : Long, multicellular filaments, straight or spiral, lacking distinct cell septa (as in the genus Arthrospira).

Color : Bluish-green (Cyanobacteria) due to the presence of the green pigment chlorophyll and the blue pigment phycocyanin.

Size : Very minute (microalgae), with filaments ranging from 50 to 500 micrometers in length and very narrow in width (3-4 micrometers).

Appearance (to the naked eye) : They appear in alkaline water bodies as a dark green biomass and are usually marketed as powders or tablets.

Figure 1: Morphological form ***(internet)***

I.3. Systematic classification

Kingdom : Bacteria.

Phyllium : Cyanobacteria.

Class : Cyanobacteria (Cyanophyceae).

Order : Nostocales.

Family : Phormidiaceae.

Genius **:** Arthrospira (the most scientifically accurate classification for commercially available species such as A. platensis and A**(Sinetova A M,2024)**

I.4. Chemical compositions

Spirulina is a microalga well known for its complex and nutritionally rich chemical composition, which makes it a valuable organism for various food, pharmaceutical, nutraceutical, and animal feed applications. The composition of spirulina is primarily characterized by its high content of proteins, lipids, carbohydrates, vitamins, minerals, and pigments, all of which contribute to its biological activities.

1) **Proteins :**

The most abundant component of spirulina is protein, which accounts for 50–70% of its dry weight. This high protein content is particularly notable because spirulina contains all essential amino acids, making it a complete protein source comparable to that found in animal products such as meat and eggs. Essential amino

acids (38.81%) include leucine (7.67%), lysine (4.37%), methionine (2.39%), phenylalanine (4.42%), threonine (4.88%), tryptophan (1.93%), and valine (6.37%). Non-essential amino acids (61.19%) such as alanine (7.52%), arginine (7.65%), glycine (5.24%), and serine (4.16%) are also present in significant amounts, further increasing the quality of the protein profile of spirulina. This makes spirulina an attractive source of dietary protein, especially for vegetarian and vegan populations.

One of the primary challenges of using spirulina as a protein ingredient, particularly at higher incorporation levels in diets, is its rigid peptidoglycan cell wall, which makes the digestibility, bio accessibility, and bioavailability of its nutrients difficult. The proteins in spirulina are often bound in protein–pigment complexes within the thylakoid membrane, making them difficult to access without adequate pre-treatment. Studies have demonstrated that mechanical and physical pre-treatments, such as bead milling, extrusion, freeze-drying, heating, microwave, and sonication, can significantly enhance the solubility and degradation of these proteins. All of these pre-treatments have advantages and disadvantages, and they are described in Table 1. Of these methods, extrusion has proven to be especially effective at disrupting the cell wall and increasing protein bio accessibility, with or without the combination of enzymes. By denaturing and aggregating key proteins like phycocyanin, extrusion facilitates greater nutrient availability for digestion. This approach may allow spirulina to be incorporated at higher levels in diets.

2) **Lipids :**

Lipids in spirulina are present in lower quantities than proteins but still play an important role in its overall chemical composition. The lipid fraction represents approximately 5–10% of the dry biomass, consisting primarily of polyunsaturated fatty acids (PUFAs). The most prominent fatty acid in spirulina is γ-linolenic acid (GLA ; 18 :3n − 6), an omega-6 fatty acid known for its anti-inflammatory properties. Other important fatty acids include linoleic acid (18 :2n-6), oleic acid (18 :1c9), and palmitic acid (16 :0). The GLA content in spirulina can vary depending on cultivation conditions but typically ranges between 1 and 2% of the total dry weight. Additionally, spirulina contains smaller amounts of omega-3 fatty acids such as α-linolenic acid (ALA ; 18 :3n-3), although these are present in much lower concentrations compared to GLA and other PUFAs.

3) **Carbohydrates :**

Carbohydrates constitute approximately 15–20% of the dry weight of spirulina. These carbohydrates are primarily in the form of polysaccharides, which serve both as structural components and as storage molecules within the cyanobacterium. Notably, spirulina contains sulphated polysaccharides that are known for their bioactive properties, including antiviral, anti-inflammatory, and immune-modulating activities. The main monosaccharides found in spirulina include glucose, rhamnose, xylose, mannose, and galactose.

These polysaccharides form complex structures, which contribute to the cell wall integrity and potentially impact the organism's bioactivities. Sulphated polysaccharides, including calcium spirulan (Ca-SP), a very unique sulphated polysaccharide, are also present in its cell wall. In addition, spirulina contains lower-molecular-weight carbohydrates, including glycogen, which serve as an energy reserve.

4) **Pigments :**

One of the defining characteristics of spirulina is its rich pigment content, which is responsible for its distinctive blue-green colour. The primary pigment in spirulina is phycocyanin, a blue pigment–protein complex that can account for up to 47% of its dry weight. Phycocyanin plays a key role in the light-harvesting complexes of photosynthesis and is also recognized for its potent antioxidant properties. Beyond phycocyanin, spirulina contains other pigments, such as chlorophyll and carotenoids, vitamin B and vitamin E. Chlorophyll is essential for photosynthesis (**maria p. spinola at all;2024**)

I.5. Pharmaceutical and cosmetic importance

Spirulina offers numerous health and beauty benefits for both men and women, most notably :

- Antioxidant and Anti-inflammatory Properties :

Phycocyanin, the main component of spirulina, is a powerful antioxidant that combats the damage caused by oxidative stress. This means it prevents the formation of harmful, inflammation-causing molecules (free radicals), thus promoting overall health and protecting the body from the damaging effects of free radicals.

- Cholesterol Reduction :

Studies indicate that spirulina may help lower total cholesterol, LDL (bad cholesterol), and triglyceride levels, while improving HDL (good cholesterol) levels. One study showed that consuming 1 gram of spirulina daily for 3 months significantly reduced total cholesterol levels.

Because of this effect, spirulina helps maintain healthy arteries, reducing the risk of heart disease and stroke.

- Immune System Support :

Spirulina is a rich source of vitamins and minerals that boost the immune system, such as vitamin E, vitamin C, and vitamin B6. It is also believed to stimulate the production of white blood cells and antibodies, which help the body fight viruses and bacteria.

Several laboratory studies have suggested that spirulina has antiviral properties against herpes, influenza, and other viruses, but these effects have not yet been proven in humans.

- Lowering Blood Pressure :

Studies have shown that consuming spirulina daily at a dose of 1–8 grams for 3 months can reduce systolic blood pressure (the top number) by 4.59 mmHg and diastolic blood pressure (the bottom number) by 7.02 mmHg, especially in people diagnosed with hypertension.

- Blood Sugar Control :

A recent study reported that taking 2 grams of spirulina daily with metformin (a diabetes medication) improved HbA1c and fasting blood sugar levels compared to using metformin alone.

- Eye Health Support :

Spirulina is rich in beta-carotene, which the body converts into vitamin A, essential for eye health. One study suggested that daily spirulina intake promotes the health of the retina and light-sensitive cells in the eye, but these findings are preliminary, and further research is needed to confirm them.

- Oral Health Promotion :

Spirulina may protect against gum disease and tooth decay. One study reported that using a mouthwash containing spirulina reduced plaque buildup and the risk of gingivitis, while another study indicated that it may reduce the risk of oral cancer in tobacco users.

- Weight Loss :

Spirulina may aid in weight loss by reducing appetite, waist circumference, and body fat percentage. It also improves blood lipid levels, partly due to its ability to decrease fat absorption in the intestines.

Spirulina also contains protein that takes longer to digest, reducing hunger. It helps prevent fat accumulation in the liver and promotes satiety, which also contributes to weight loss.

- Reduced Cancer Risk :

Spirulina contains potent antioxidants that reduce chronic inflammation in the body, which is believed to be a major risk factor for cancer and other diseases.

This is likely due to phycocyanin, a compound that reduces inflammation and inhibits tumor growth. However, these findings are still preliminary, and further research is needed to confirm them.

- Allergy Relief :

Nasal allergies occur in some people when they are exposed to dust, pollen, or pet dander. One study reported that spirulina may relieve the following nasal allergy symptoms :

- Congestion.

- Runny nose.
- Sneezing.
- Itching.

Another study compared the effects of spirulina to the allergy medication cetirizine, showing that allergic rhinitis symptoms improved more significantly with a daily intake of 2 grams of spirulina. However, these results are still preliminary.

- Improved Skin Health :

Spirulina may be beneficial for skin health, as it is believed to help :

- Boost collagen production and the cells responsible for skin repair.
- Reduces skin inflammation and damage.
- Protects against UV damage.
- Prevents acne breakouts (**lama at all; 2024)**

CHAPTER II :

Cosmetic products and the skin

II.1 General information about beauty products (serums)

Cosmetics are natural or synthetic toiletry products that are used to maintain hygiene and include externally applied products used to enhance appearance. This class includes dental products, bath supplies (e.g., bubble baths, body washes, and bath beads), powders, lotions, lipsticks, perfumes, colognes, shampoos, depilatories, and hair coloring/waving products. Most of these products contain alcohols, aromatic hydrocarbons, perborates, and anionic and nonionic surfactants (**Manayi, A 2014, saeidnia.s,2014)**

II.1.2 Definition of serums

Face serums are concentrated, lightweight skincare products made with smaller molecules than other skincare products, such as creams and lotions, allowing them to penetrate the skin more effectively and deeply.

These often contain active ingredients that target specific skin concerns, such as wrinkles, dark spots, or dryness(**dr.andre mahns,2026)**

II.1.3 Key Serum Characteristics and Features

High Concentration: Contains very high concentrations of active ingredients (such as vitamins and hyaluronic acid) compared to regular creams.

Light and Fast Absorbing: Features a very light, watery or gel texture, allowing it to glide smoothly onto the skin and absorb without leaving any greasy residue.

Deeper Penetration: Thanks to its fine molecular structure, it penetrates the surface layers to reach deep into the skin.

Targeted Treatment: Each serum is designed to address a specific concern, such as acne, pigmentation, wrinkles, or dryness**(Mankar S;2024)**

II .1.4.The essential component of face serums

Hyaluronic Acid: The Ultimate Moisturizer

Hyaluronic acid is a common ingredient in hydrating serums, known for its exceptional ability to retain moisture. It helps hydrate the skin and give it a plump, youthful appearance, making it especially suitable for dry and aging skin. This ingredient can hold up to 1,000 times its weight in water, ensuring long-lasting hydration. Adding hyaluronic acid to moisturizer formulas guarantees effective hydration benefits for all skin types.

Vitamin C: Skin Brightening and Anti-Aging Benefits

Vitamin C is a powerful antioxidant that protects the skin from free radical damage, boosts

collagen production, and reduces the appearance of dark spots, fine lines, and other signs of aging. This key ingredient in facial serums helps brighten and even out skin tone. Effective serum formulas are designed to maximize the benefits of vitamin C, making it a staple in the anti-aging beauty serum industry.

Niacinamide: A Multi-Benefit Ingredient for Sensitive Skin

Niacinamide, or vitamin B3, is a powerful ingredient that offers multiple benefits for the skin. It helps regulate oil production, reduce redness and blemishes, and improve the skin's barrier function. It also minimizes the appearance of pores and fine lines, making it a valuable addition to facial serums. Using niacinamide in skincare products provides comprehensive solutions for improving and purifying the skin, suitable for various skin types and concerns.

Peptides

Peptides are short chains of amino acids that are the building blocks of proteins like collagen and elastin, essential for maintaining skin structure and elasticity. Incorporating peptides into facial serums can help stimulate collagen production, reduce wrinkles, and improve skin firmness. Advanced peptide technologies in lightweight serum formulations ensure effective anti-aging benefits in cosmetics.

Plant Extracts:

Plant extracts and antioxidants such as aloe vera, green tea, and chamomile lend soothing and anti-inflammatory properties to facial serums. These natural ingredients help calm the skin, reduce redness, and provide antioxidant protection. Committed to sustainable and environmentally friendly practices, a variety of plant extracts are incorporated into the cosmetic manufacturing processes to produce gentle yet effective serums suitable for sensitive and oily skin(**Arminak2024)**

II .1.5.Types of face serums

1.The Oil Serum

An oil serum contains natural plant oils such as argan, jojoba, or rosehip oil.

It deeply moisturizes and nourishes the skin while strengthening the skin barrier, making it ideal for dry skin.

2. The Gel Serum

Gel serum has a lightweight, refreshing texture that absorbs quickly without leaving a greasy feel.

It hydrates the skin effectively and is especially suitable for oily and combination skin types.

3. The Water-Based Serum

This type is mainly made with water and hydrating ingredients such as hyaluronic acid and

glycerin.

It provides light yet deep hydration and is suitable for all skin types, especially sensitive and oily skin.

4. The Emulsion Serum

An emulsion serum combines water and oil in a balanced, lightweight formula.

It offers both hydration and nourishment while helping active ingredients penetrate the skin more effectively.

5. The Pressed Balm Serum

This serum has a rich balm-like texture and contains nourishing butters and oils.

It provides intense hydration and is best suited for very dry or damaged skin(***Mankar S,2024)***

II.2 Skin Overview

The skin is the body's largest organ and performs many vital functions, including

Protecting the body from injury

Regulating body temperature

Maintaining water and electrolyte balance

Sensing both painful and pleasant stimuli

It also participates in the production of vitamin D

The skin stores essential chemicals and nutrients within the body; simultaneously, it provides a barrier preventing harmful substances from entering the body and protects against the damaging effects of ultraviolet radiation from the sun. Furthermore, skin color, texture, and folds contribute to each individual's unique personality traits. Anything that interferes with skin function or causes changes in its appearance can have significant impacts on physical and mental health.

Many skin problems remain confined to the skin itself. However, the skin can sometimes provide information about systemic disorders. Consequently, doctors must consider several potential diseases when evaluating skin problems. They may need to order blood tests or other laboratory examinations to rule out internal diseases in people with skin problems(**Benedetti.j,2026)**

II . 2.1. The skin consists

II .2.1.1.The epidermis

It is the thin outer layer of skin, acting as a protective barrier against germs and external elements, and preventing water loss. It contains cells that produce keratin and melanin, which

are responsible for skin color and protection from ultraviolet radiation.

II .2.1.2.The dermis

It is the middle and thickest layer of the skin, containing blood vessels, nerves, sweat and sebaceous glands, and hair follicles. It is responsible for sensation, nourishing the skin, regulating temperature, and giving the skin elasticity and strength.

2.2.3.The subcutaneous fat layer

It is the deepest layer of the skin and is composed mainly of fat. It insulates the body from heat and cold, stores energy, protects internal organs, and anchors the skin to the underlying tissues(**Benedetti.j,2026)**

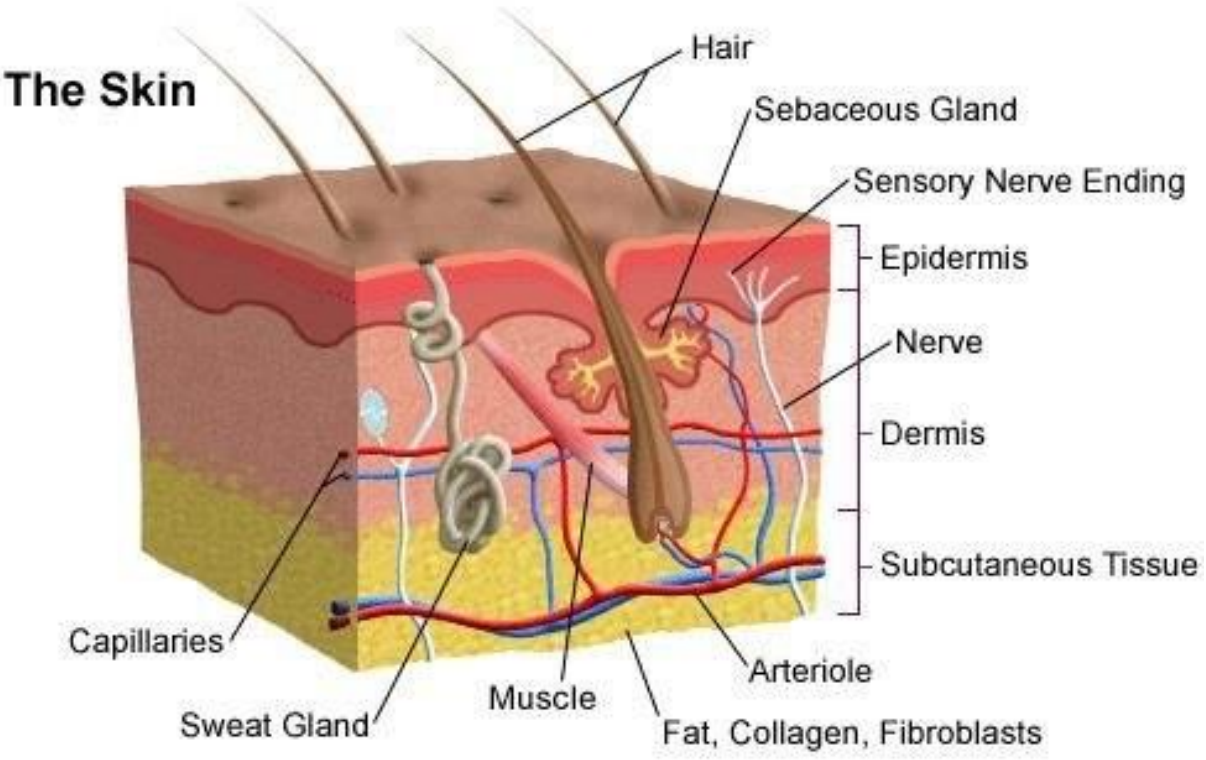

Figure 2: the different layers of the skin(https://www.faceslaser.com)

II.3 Common skin diseases and conditions

▪ Eczema : A chronic skin condition, often of allergic origin, characterized primarily by red patches that cause intense itching.

▪ Wound : An opening or tear in the tissue caused by treatment such as an injury or a cut. There are different types of wounds, whether caused by a sharp instrument or a pointed object.

▪ Wrinkle : A furrow or fold marking the skin.

▪ Scarring : is the physiological process of tissue repair. The scar is the final result and is often a source of concern for the patient.

▪ Stretch marks : These are linear, atrophic depressions in the skin tissue, with a smooth or folded surface and a soft consistency. Their color is often purplish-red at first, becoming pearly white in the later stages.

▪ Cellulite : Excessive development of fatty deposits in the hip and thigh area, more common in women. Cellulite gives the skin a grainy, dimpled appearance and a soft, flabby consistency(2025,

SECOND PART

Experimental study

CHAPTER II

Materials & Methods

II / Materials and Methods

The aim of this study is to develop a cosmetic facial serum based on spirulina extracts and to evaluate its biological efficacy . to achieve this goal , the experimental part was conducted in the laboratory of the faculty of natural and life sciences university of EL Oued .

II.1 Biological Materials

The spirulina used in this study is a fine , bluish - green powder extracted from the cyanobacterium *Arthrospira platensis* (nordstedt 1884). It was obtained from a certified supplier this strain is cultivated in open ponds under controlled climatic conditions without the use of pesticides or herbicides .

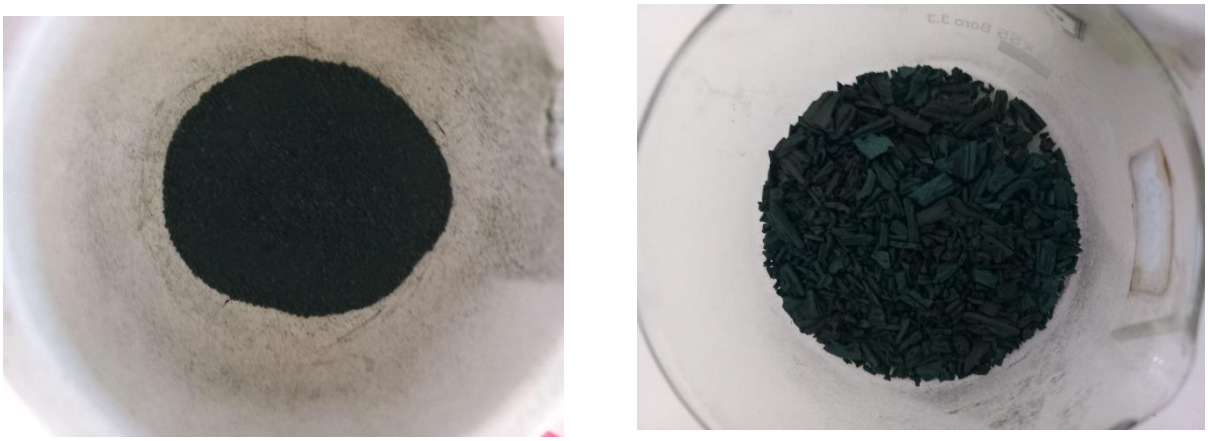

Figure 03: Spirulina powder and flakes

II.2 . Methods

II.2.1.Preparation of spirulina extracts

approximately 2g of powdered plant material are macerated in 50ml of each solvent separately (distilled water and ethanol) for 15 hours under agitation the filtrate is then stored

II.2.2 Defatting

•Weigh 25 g of the powder into a 500 mL glass flas.

* Add 125ml of ethanol and 125ml of acetone .

•Filter the mixture using Whatman No. 1 paper.

•Repeat the same treatment on the precipitate with 250 mL of fresh ethanol.

•Air dry the precipitate for 24 hours, then in a 40°C oven for 3 hours.

II.2.3 Protein Extraction

•Take 5 g of the defatted powder.

•Add 100 mL of distilled water.

•Stir for 2 hours at room temperature.

•Centrifuge the solution at 4000 rpm for 20 minutes.

•* Collect the greenish-blue filtrate (containing albumins and phycocyanin). Store the extract in a refrigerator at 4°C

II.2.4 Rapid pre- Purification

*Simple Double Filtration

•Pass the extract through Whatman No. 1 filter paper twice to remove suspended particles.

II.2.5 Lyophilization _ Simplified Final Step

•Place the resulting solution into suitable glass bottles.

•Freeze the bottles at -20°C for 4 hours (or overnight).

•Directly dry the samples in a lyophilizer for 24–48 hours.

•Obtain a dry powder (white to light green).

•Store the powder in tightly sealed dark glass bottles at -20°C until ready to use.

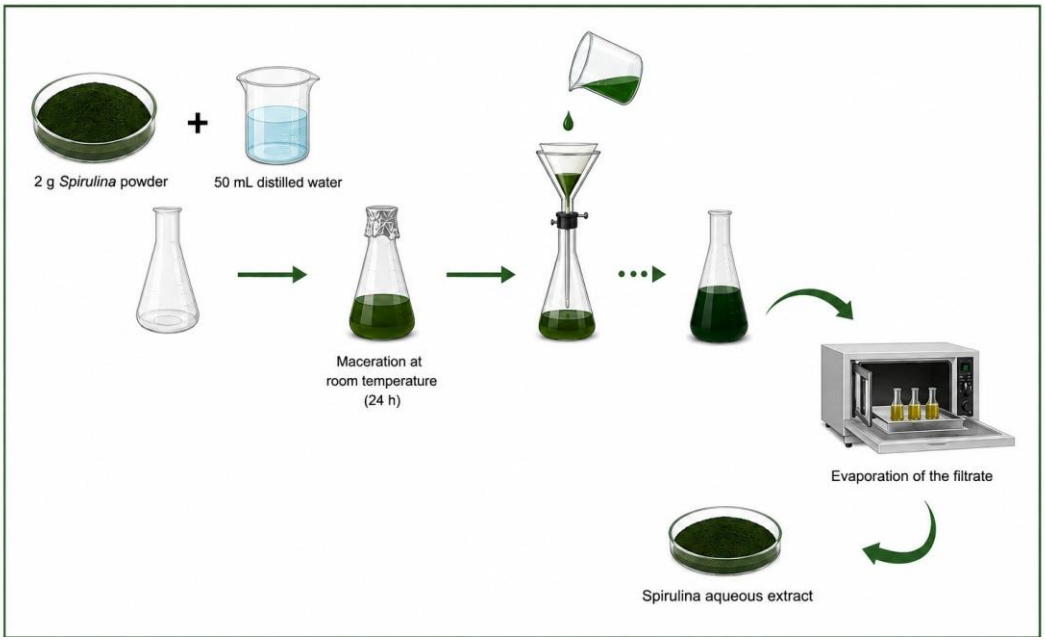

Figure 04 : Preparation of spirulina aqueous extract

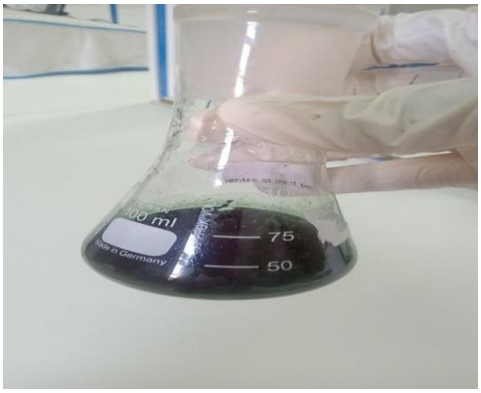

Figure 05: Extraction

II.3. Preparation of a cosmetic serum based on an active spirulina extract

Two formulations were prepared for each skin type (dry, oily).

II.3.1 Oily skin

The First formula

Table 01: compounds of the first formul

INGREDIENTS	FUNCTION	
spirulina extracts	Active ingredient	3%
Distilled water	Aqueous carrier solvent	70%
Glycerin	A moisturizer	2%
Aloe Vera gel	Soothing	8%
Vitamin C	Collage production	0.3%
Panthenol (provitamin B5)	Moisturizing , soothing , healing	1%
Xanthan gum	A thickener	0.8 %
Preservative(phenoxyethanol)	Microbiological protection	3%

* Manufacturing process

First , prepare the xanthan gum 0.8% beforehand by dissolving it in distilled water70% and letting it stand for 15 minutes to ensure complete dissolution . after 15 minutes , add the remaining ingredients : aloe Vera gel 8% , glycerin 2% , and panthenol 1%.blend a magnetic blender until smooth . then add the spirulina extract 3% , Vitamin C 0.3% , and preservative 3%. Pour the mixture into a dark- colored bottle and store it away from sunlight.

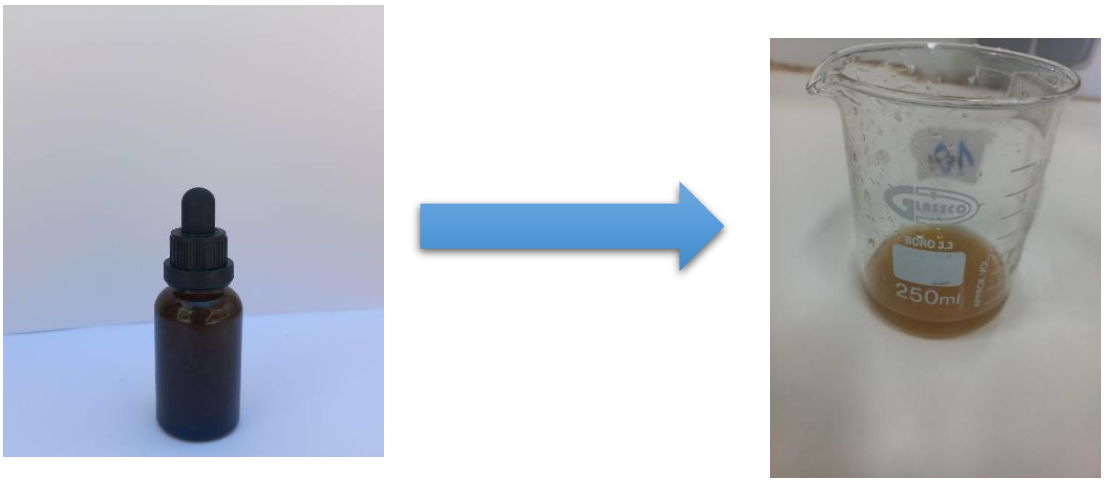

Figure 06: The final product of the first combination (oily skin)

* The second formula:

Table 02：components of the second formula

INGREDIENTS	FUNCTION	
spirulina extracts	Active ingredient	3%
Distilled water	Aqueous carrier solvent	70%
Glycerin	A moisturizer	8%
Panthenol (provitamin B5)	Moisturizing , soothing , healing	1%
Vitamin E	Antioxidant , skin protection	2%
Hyaluronic Humectant	Attracts moisture	0.3%
Aloe Vera gel	Soothing	8%
Preservative(phenoxyethanol)	Microbiological protection	3%

* Manufacturing process

First , prepare the hyaluronic acid gel by combining distilled water 70% and hyaluronic acid 0.3% . mix the mixture using a magnetic blender (100 -150 PRM) for two minutes and let it stand for two hours to allow the gel to form . Add the remaining ingredients : Aloe Vera gel8% , glycerin2% , panthenol 1% , and spirulina extract3% . mix well , then add Vitamin E 2%and the preservative3% .

Package the mixture in a dark – colored bottle and store it away from sunlight .

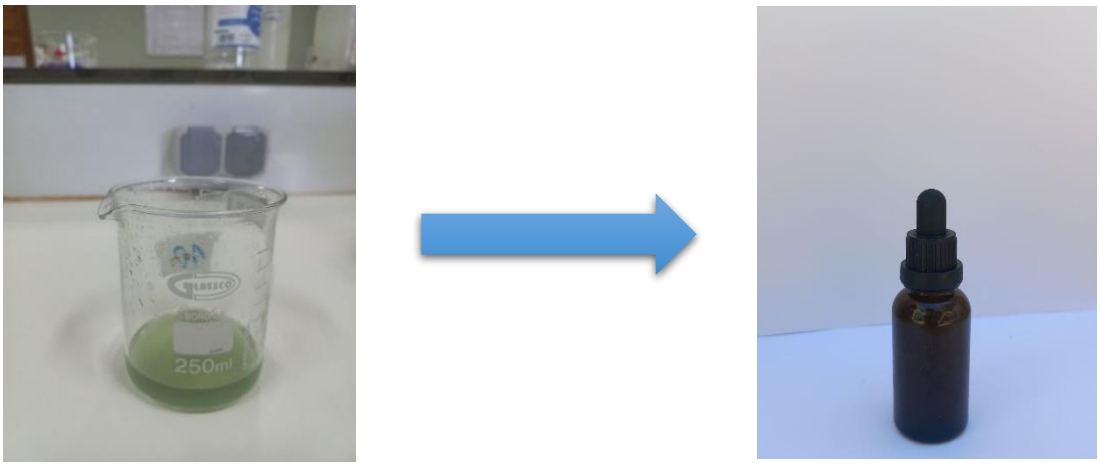

Figure 07: the final product of the second combination(oily skin

II.3.2 Dry skin (the only difference is in the percentage of extract)

Table 03: components of the two formuls

INGREDIENTS	FUNCTION	
spirulina extracts	Active ingredient	3%
Distilled water	Aqueous carrier, solvent	70%
Glycerine	A moisturizer	2%
Aloe vera gel	Soothing	8%
Jojoba oil, avocado oil, grapeseed oil	Carrier and nourishing	6%,9%,12%
Beeswax	Emulsion stabilizer	0.5 %
Emulsion B	Emulsion stabilizer	1.5%
Xanthan gum	a thickener	0.8%
Vitamin E	An antioxidant	2%
Preservative(phenoxyethanol)	Microbiologic Protection	3%

* Manufacturing process

The aqueous phase, containing distilled water 70%, glycerine, and aloe vera gel 8%, is heated to 75°C. similarly, the oil phase, composed of oils, emulsion B21.5%, and beeswax0.5%, is heated to 80°C. the aqueous solution is then poured onto the oil solution while stirring slowly with a magnetic mixer for 3 minutes. the speed is then increased to 1500 RPM until the two phases are homogeneous. after reducing the temperature to 40°C, vitamin E 2%, the extract 3%; the preservative3%, and finally the xanthan gum0.8% previously dissolved in glycerine 2% are added. the mixture is then packaged in a dark -colored bottle and stored away from sunlight.

Figure 08: Serum formulation; water phase heating stage.

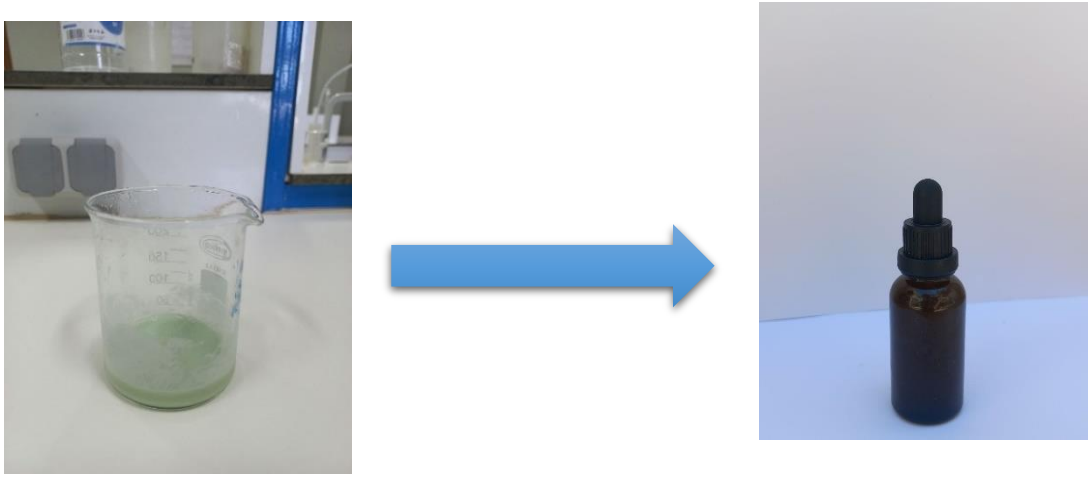

Figure 09: the final product of the first combination (dry skin)

II.3.3. Controls Performed on the serum

II.3.3.1. PH Measurement

The pH is measured using pH paper and a pH meter for 1 g of serum.

II.3.2.2. Stability assessment

Centrifugation test

A quantity of serum is placed in a centrifuge tube set at 3000 rpm for 30 minute to detect any phase separation.

II.4. Animal Experimentation Protocol (in vivo study)

II.4.1. Evaluation Of Four Spirulina Based serums on dry and oily skin models

II.4.1.1. Animals and Housing

Twenty-four adult female white mice, with an average weight of 150 grams, were obtained and housed in the experimental animal unit of the Department of Molecular and Cellular Biology, Faculty of Natural and Life Sciences, University of El Oued, Algeria. The animals were acclimatized for one month under the same laboratory conditions: a 12-hour light/12-hour dark cycle, 64 ± 2% relative humidity, and a room temperature of 19 ± 1°C. Standard mouse food and unlimited access to tap water were provided throughout the experiment. The mice were cared for and handled during all experimental procedures in accordance with the practices recommended by the local ethics committee.

II.4.1.2. Experimental Design

The mice were randomly divided into 6 groups each group containing 4 mice (N=4).

Before any intervention, all animals were left for 30 days under the housing conditions described above to allow them to acclimatize to the environment and the handlers.

During this period, the rats were handled daily (gentle grasping, bedding change) to minimize stress during the experiment

II.4.1.3. Skin Preparation (Hair Removal)

On day 30, the dorsal hair of each rat was carefully shaved using a new sharp razor blade, taking care to avoid skin cuts or irritation. the shaved area was cleaned with warm sterile water, gently dried with sterile gauze, and left for 24 hours to confirm absence of inflammation.

Figure 10: shaving stage

II.4.1.4. Induction of Skin Disorders (1week)

Skin disturbances were induced twice daily (morning and evening) for seven consecutive days.

Skin dryness was induced (groups G2 and G3, and in mice with dry skin in group G6)

SLS solution with distilled water was applied to the dorsal skin for 30 seconds twice daily for seven days.

Sebum secretion was induced (in groups G4 and G5, and in mice with oily skin in group G6)

Virgin olive oil was applied evenly to the shaved dorsal skin twice daily for seven days.

Group G1(the negative control group) received no stimulation and was left with healthy skin

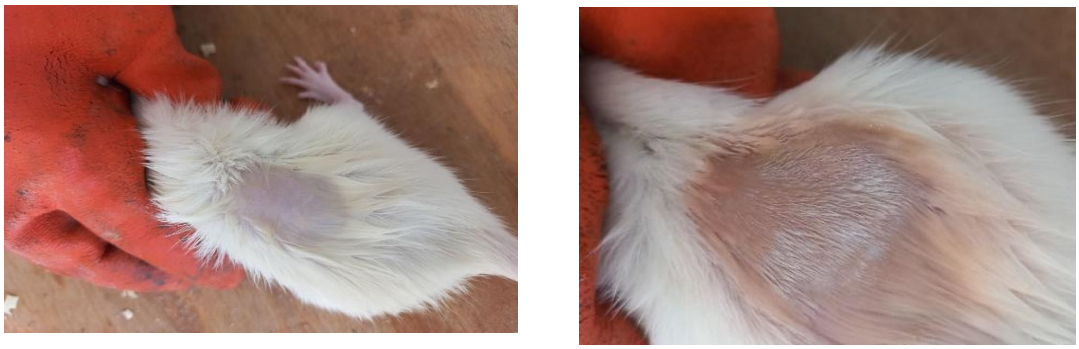

Figure 11: Nursing stage.

II.4.1.5. Treatment phase (1 week)

Immediately after the induction week, treatment was applied twice daily (morning and afternoon) for 7 consecutive days, without further induction. each application consisted of 50 up of the respective product, gently spread over the shaved area using sterile gloved fingers.

Gouge treatment applied

* G1 50uL sterile physiological saline (healthy skin)

* G2 50uL dry skin serum-formulation A (1% spirulina).

* G3 50uL dry skin serum-formulation B (1% spirulina).

* G4 50uL oily skin serum – formulation C (1% spirulina).

* G5 50uL oily skin serum – formulation D (1% spirulina).

* G6 2 dry -induced rats: 50uL place do serum (spirulina -free) 2 oily -induced rats: 50uL place do serum (spirulina -free)

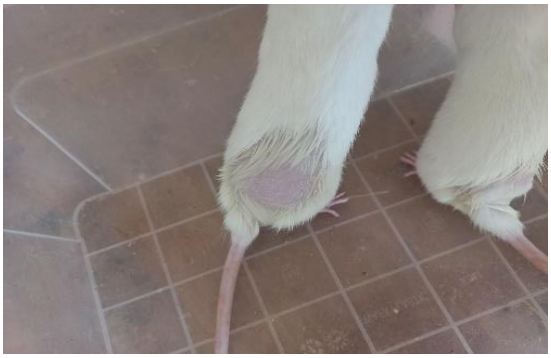

Figure 12: Treatment phase.

II.5 Sample Preparation

II .5.1. Sacrifice and blood collection:

After treatment, the mice were decapitated under mild anaesthesia using 94% chloroform. the samples were transferred to EDTA-containing tubes and SAC tube, which had been previously numbered and labelled for each mouse. The resulting serum was centrifuged at 2500rpm for 10 minutes at 20°C and stored until needed.

II.5.2. Preparing a tissue sample:

The area where the treatment was applied was excised and fixed in formaldehyde (10%) for histopathological examination.

II -6 Hematological and Biochimical parameters analysis

II .6.1 Hematological Analysis Protocol and Principle

Blood samples were collected from experimental animals into EDTA tubes to prevent coagulation. The samples were gently homogenized and analyzed using an automated hematology analyzer according to standard laboratory procedures. The principle of this analysis is based on electrical impedance and flow cytometry techniques, which allow the counting and differentiation of blood cells according to their size, volume, and physical properties. The evaluated parameters included red blood cells (RBC), white blood cells (WBC), hemoglobin concentration (Hb), hematocrit (HCT), and platelets (PLT). The obtained results were compared with normal physiological reference values to assess the health status of the animals.

II .6.2Renal Biochemical Analysis Protocol and Principle

Blood samples intended for renal biochemical analysis were collected in dry tubes and

centrifuged at 3000 rpm for 10 minutes to obtain serum. The serum was used for the determination of urea, creatinine, and uric acid levels using commercial diagnostic kits according to standard laboratory protocols. The principle of these assays is based on enzymatic colorimetric reactions measured spectrophotometrically. Urea determination is based on the hydrolysis of urea by urease producing ammonia. Creatinine determination is based on the Jaffé reaction, in which creatinine reacts with alkaline picrate to form a colored complex. Uric acid determination is based on the oxidation of uric acid by uricase producing hydrogen peroxide, which reacts with chromogenic substrates in the presence of peroxidase to generate a colored compound. The intensity of the color formed is directly proportional to the concentration of each parameter in the serum sample.

II-7 Histopathological Study of Skin Tissue

1/ A portion of skin was excised from euthanized mice.

2/ These sections were placed in a fixative solution (10% formaldehyde) until the slides were prepared for analysis.

3/ The samples were dried using an ascending series of ethanol solutions (60%, 70%, 80%, and 100%), cleaned with xylene, and then immersed in paraffin wax. Sections 4–6 μm thick were prepared

4/ from the paraffin wax casts using a Histoline rotary microtome (Thermo Scientific (McCorn HM 325)). Hematoxylin and eosin were used for staining. Using a light microscope, histopathological observations were performed. This work was carried out inside the anatomy laboratory of al- wefaq clinic

II- 8 Microbiological Analysis Procedure for Oils

The microbiological analysis was performed to evaluate the microbial quality and safety of the oil samples according to the Algerian microbiological standards. First, 10 mL of the sample were aseptically added to 90 mL of Tryptone Salt Water (TSE) in order to obtain the stock suspension (10^{-1} dilution). The mixture was homogenized for 10 minutes to ensure proper dispersion of microorganisms. Subsequently, 1 mL of the prepared dilution was inoculated onto different selective culture media depending on the targeted microorganism. Total aerobic mesophilic germs (GA) were cultured on Plate Count Agar (PCA) using the pour plate method and incubated at 30°C. Escherichia coli (EC) was inoculated on BCP medium and incubated at 44°C. Yeasts and molds (LM) were cultured on Sabouraud agar and incubated at 25°C. For Staphylococcus aureus (St), four drops of the suspension were spread on the selective medium using a sterile glass spreader and incubated at 37°C. In addition, Pseudomonas aeruginosa (Pseud) was inoculated on its selective medium and incubated at

22°C. After incubation, the microbial growth was observed and colony counts were recorded and compared with the standard microbiological limits.

Culture medium used for yeasts and molds. The operation was carried out inside al Shahabi lab.

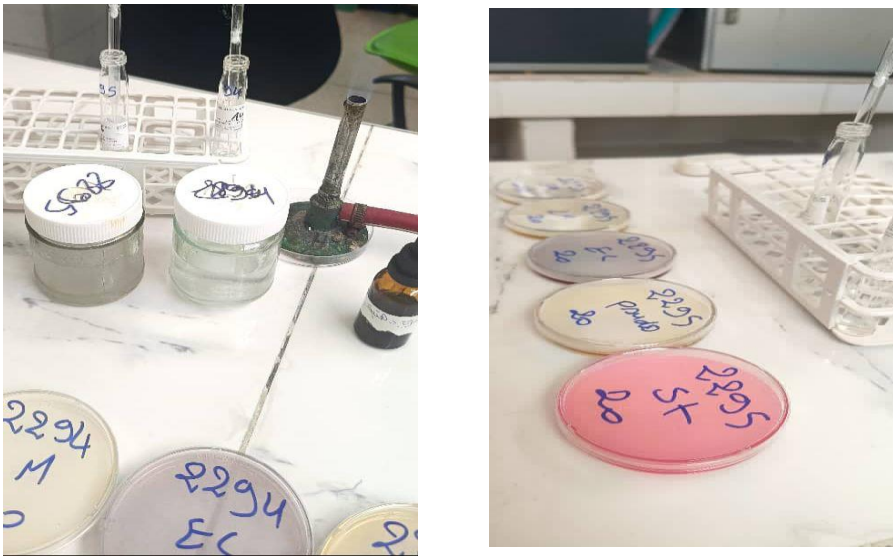

Figure13: Evaluation the antimicrobial activity of the product

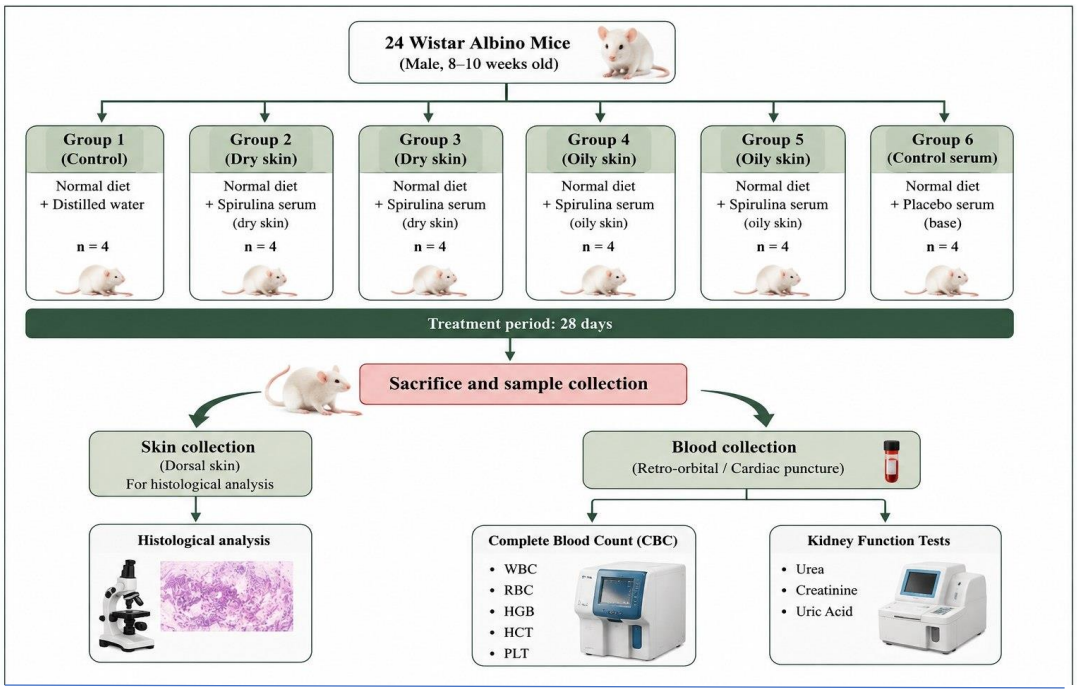

Figure 14: summary scheme of the experimental protocol.

Chapter III

Results & discussion

III. Results and discussion

III. 1 Defatting Efficiency:

A defatting protocol using 96% ethanol was applied to 25 grams of raw spirulina powder. After double processing and drying, 21.25 grams of defatted powder were obtained, resulting in a defatting efficiency of 15%.

This result indicates that the fat content in the spirulina sample used is approximately 15% of its dry weight. This value is consistent with what has been reported in the scientific literature, where the fat content in spirulina typically ranges between 5% and 15%, varying according to the algal strain and cultivation conditions.

Defatting is essential in this study for two main reasons:

• Fat can interfere with subsequent protein extraction, as it makes the powder hydrophobic, thus reducing extraction efficiency.

• Fat is susceptible to oxidation and rancidity over time, which can affect the stability of the final cosmetic product.

Previous studies have shown that ethanol treatment is an efficient and simple method for removing lipids from microalgae biomass compared to other chemical methods that may consume large quantities of solvents. Therefore, lipid removal is a crucial step for improving the purity of protein extracts and extending their shelf life.

Figure15: defatting efficiency

III .2 Protein Extraction Yield

After fat removal, proteins (albumins) were extracted from 5 grams of defatted powder using 100 ml of distilled water. The extract was then purified and lyophilized, yielding a final dry protein powder weight of 2.1 grams, resulting in a final extraction yield of 8.4% (calculated relative to the initial weight of crude spirulina of 25 g).

III-2-1 Protein Extraction Efficiency

• The results indicate that protein extraction using distilled water at room temperature for 2 hours is efficient, recovering approximately 8.4% of the total dry mass as concentrated protein.

• This yield is considered acceptable compared to other conventional extraction methods. In a 2015 study, a protein yield of up to 60.7% (w/w) was achieved using optimized extraction conditions involving a basic pH (pH 11.38) followed by precipitation at an acidic pH (pH 4.01). In contrast, the conventional basic extraction method showed a relatively low yield and high solvent consumption.

In comparison, the yield obtained in this study (8.4%) is crude protein, which is lower than the yields achieved using the optimized basic conditions. This is expected for two reasons: first, only albumins were extracted (not all proteins), and second, only water was used as the solvent without any additional chemical or enzymatic treatment.

In a more recent study (2025) using a basic solute and acid precipitation method, a protein isolate yield of 280.36 mg/g, or approximately 28%, was achieved.

Another study indicated that water extraction can achieve high protein concentrations of up to 92% in the concentrated fraction, demonstrating that water is an effective solvent for soluble proteins in spirulina.

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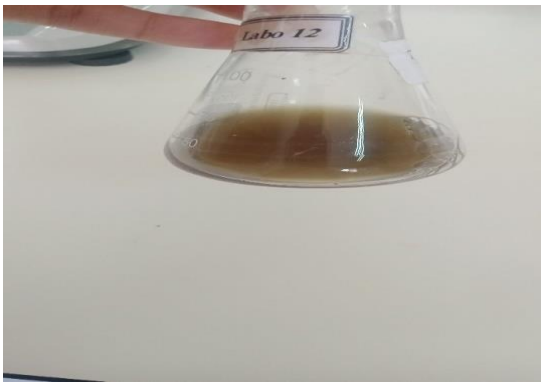

Figure16: protein extraction results

III.3 Characteristics of reformulated products

Organoleptic characteristics of the products developed

The evaluation of the cream's sensory properties, essential for product acceptability. The cream and balm based on amaranth extract were successfully formulated.

III.3.1. Developing a cosmetic product

incorporating plant extracts requires a thorough understanding of the characteristics and benefits of their phytochemical compositions, as well as mastery of preparation techniques that meet rigorous standards of precision.

Figure17: sample of e reformulated beauty products

III -3-2. PH Measurement:

Table 04: Results of ph measurement

Type of skin	Oily skin	Dry skin
Ph	5,06	5,25

The pH measurements showed that the serum for dry skin had a value of 5.25, while the serum for oily skin had a value of 5.06. These slightly acidic values fall within the normal skin pH range (4.5–6.5), indicating that both formulations are suitable for topical use and are skin-compatible.

The comparison between the two serums shows that the oily skin serum is slightly more acidic than the dry skin serum. This is considered beneficial from a cosmetic perspective, as a mildly acidic environment helps reduce the growth of acne-causing bacteria and regulates sebum production, whereas a slightly higher pH in the dry skin serum supports skin hydration

and helps maintain the skin barrier.

These results are consistent with previous studies, which reported that skincare products with a pH close to natural skin pH improve skin barrier function and reduce irritation. Other studies also highlighted that a slightly lower pH in products designed for oily skin can help inhibit microbial activity associated with acne without negatively affecting the skin.

III-3-3 Evaluation of serum stability

Centrifugation test

After centrifugation for 30 minutes, we observe the separation of the components.

This phenomenon can be explained by the density difference between the oily and aqueous phases, as well as the limited effectiveness of the emulsifying agent in maintaining homogeneity under mechanical stress. It may also result from inadequate mixing during preparation or from the nature of the bioactive compounds extracted from spirulina.

III -4 Animal Experimentation Protocol (in vivo study)

III-4-1 Hematological parametres

Table 05: bold test results

Parameters	**Wbc cell(103/ul)**	**Rbc 10^6/µL)**	**Hb (g/dL)**	**Plt (10^3/µL)**	**Hct %**
G2/g4	5,4	6,56	13,2	734	**41,2**
Reference	4.0_10.0	4.0_6.0	11.0_16.0	100_400	**37.0_50.0**

In the complete blood count (CBC), the white blood cell (WBC) count was approximately 8.4 × 10^9/L, which falls within the normal reference range (4.0–10.0 × 10^9/L), indicating the absence of acute inflammation or severe infection. However, the lymphocyte percentage was 76.6%, compared to the normal range (20–40%), a significant increase that may indicate immune system stimulation or an immune response resulting from exposure to the studied substance. Conversely, the granulocyte percentage was 15%, compared to the normal range (50–70%), a decrease that may be related to an imbalance between different types of white blood cells.

As for red blood cells (RBCs), the count was 6.56 × 10^{12}/L compared to the reference range (4.00–6.00 × 10^{12}/L), indicating a slight increase. Hemoglobin (HGB = 13.2 g/dL) and hematocrit (HCT = 41.2%) remained within approximately normal limits, suggesting the absence of overt anaemia in the mice.

However, the RBC indices showed a marked decrease in mean corpuscular volume (MCV =

62.7 FL) compared to the normal range (82–95 FL), as well as a decrease in mean corpuscular hemoglobin (MCH = 20.1 pg.) compared to normal values (27–31 pg.). This indicates the presence of relatively small, hypochromic red blood cells, which may be related to metabolic changes or a partial deficiency in hemoglobin synthesis. The red blood cell size variation coefficient (RDW-CV = 19.8%) was also elevated compared to the reference range (11.5–14.5%), indicating anisocytosis (variability in red blood cell sizes).

Platelet count was elevated (PLT = 734 × 10^9/L) compared to the normal range (100–400 × 10^9/L), which may reflect a defensive response or stimulation of bone marrow activity. Platelet hematocrit (PCT = 0.497%) was also elevated compared to the normal range (0.108–0.282%), confirming increased platelet activity. Conversely, the mean platelet volume (MPV = 6.8 FL) was slightly lower than the normal range (7.0–11.0 FL), which may indicate a predominance of small platelets resulting from increased production.

- Blood tests showed no statistically significant differences between the group using spirulina serum and the control group in terms of red blood cell count (RBC), hemoglobin (Hb), and hematocrit (Hct). This indicates that the serum did not cause anemia or changes in blood viscosity during the trial period.

  Furthermore, no abnormal increase in white blood cell (WBC) or platelet (PLT) counts was observed compared to the control group, ruling out a systemic inflammatory response or effect on the bone marrow.

  These results are consistent with previous studies that confirmed that topical application of spirulina algae (at concentrations up to 2%) does not lead to clinically significant systemic absorption and is therefore safe for blood components (**El-Baky et al., 2010; Silva et al., 2017).**

III -4-2. Biochemical parameters

Table 06 Biochemical Parameters (Blood Glucose, Urea, and Creatinine) of Rats Treated with Spirulina-Based Serum

Séquence	Glycémie (g/l)	Urée (g/l)	Créatinine (mg/l)
Tableau 1	0.58	0.37	7.00
Tableau 2	0.66	0.32	5.90
Tableau 3	0.76	0.30	6.80
Tableau 4	0.71	0.31	7.10
Moyenne	**0.00**	**0.00**	**0.00**
Coefficient (R^2)	**0.68**	**0.69**	**0.08**

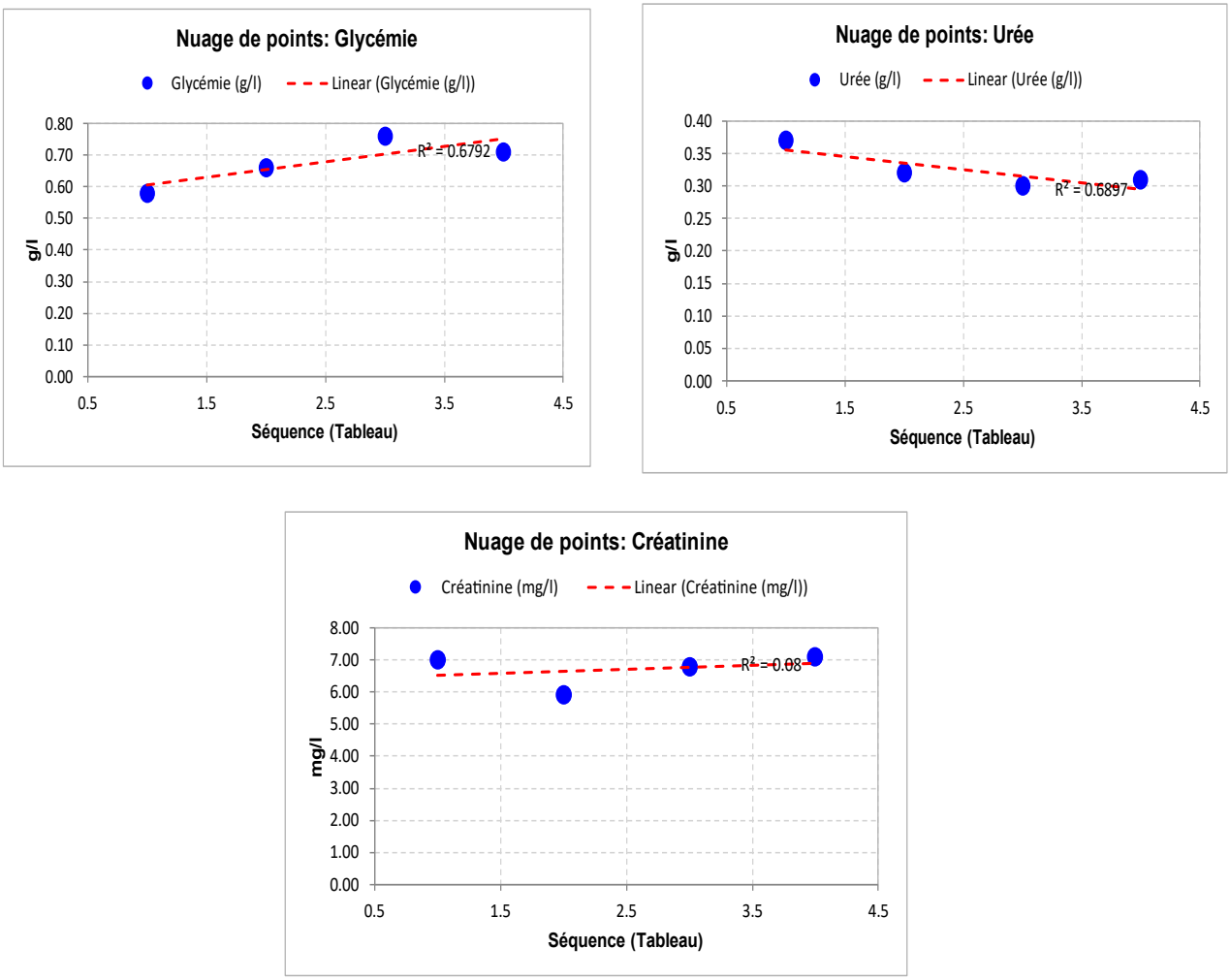

Figure18 Graphical Representation of Blood Glucose, Urea, and Creatinine Levels in the Experimental Groups

Les résultats des analyses biochimiques rénales montrent que les concentrations d'urée ont varié entre 0,30 et 0,37 g/L, tandis que les valeurs de créatinine se situaient entre 5,90 et 7,10 mg/L. Les moyennes obtenues étaient respectivement de 0,33 g/L pour l'urée et de 6,70 mg/L pour la créatinine. Ces valeurs demeurent dans les limites physiologiques normales rapportées chez le rat et ne révèlent aucune perturbation notable de la fonction rénale. L'absence d'augmentation significative de l'urée et de la créatinine suggère que l'application du sérum formulé à partir de l'extrait actif de Spirulina n'a entraîné aucun effet néphrotoxique. En effet, ces deux paramètres sont largement utilisés comme indicateurs de l'intégrité et de la capacité fonctionnelle des reins ; leur élévation est généralement associée à une altération du débit de filtration glomérulaire ou à des lésions rénales. Les résultats obtenus dans la présente étude sont en accord avec ceux rapportés par **Chamorro et al. (2002),** qui ont démontré la faible toxicité de la Spirulina et son innocuité vis-à-vis des principaux organes. De même**, Belay (2002)** a souligné que cette microalgue ne provoque pas de modifications significatives des paramètres biochimiques liés aux fonctions rénales. Cette stabilité pourrait également être attribuée aux propriétés antioxydantes de la Spirulina, riches en

phycocyanine, caroténoïdes et composés phénoliques, capables de protéger les tissus contre le stress oxydatif **(Wu et al., 2016).** Ainsi, les résultats obtenus confirment la bonne tolérance biologique du sérum à base de Spirulina et soutiennent son potentiel d'utilisation comme produit cosmétique sûr.

III- 5. Histopathological observation

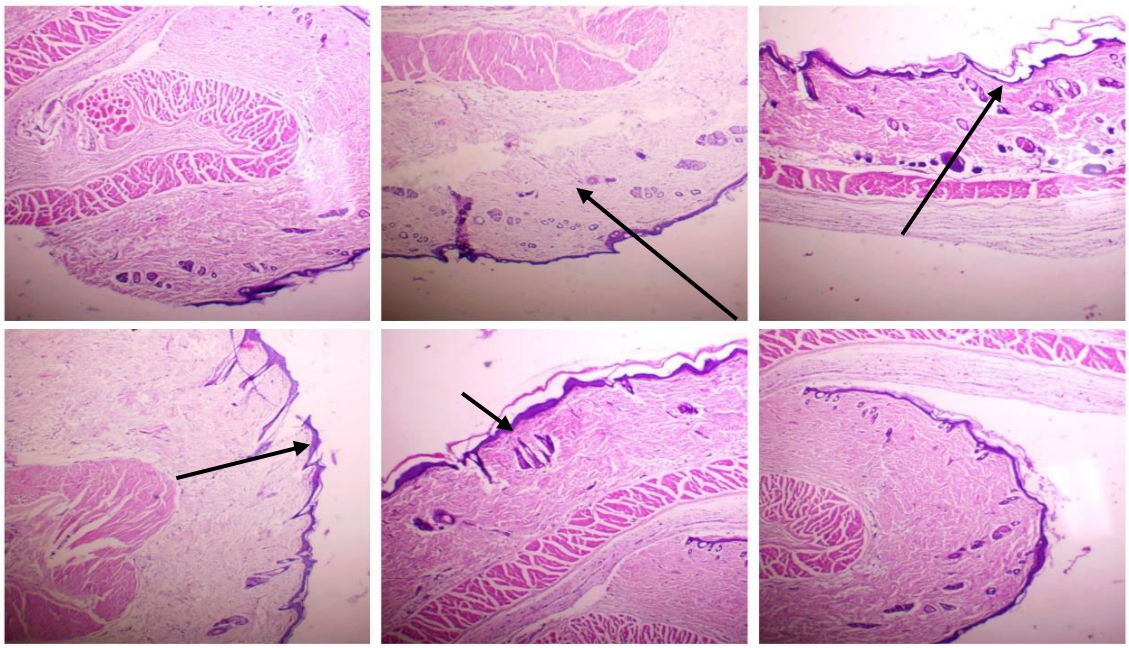

Figure 19: Microscopic images of a piece of dry epidermal skin (Composition 1)

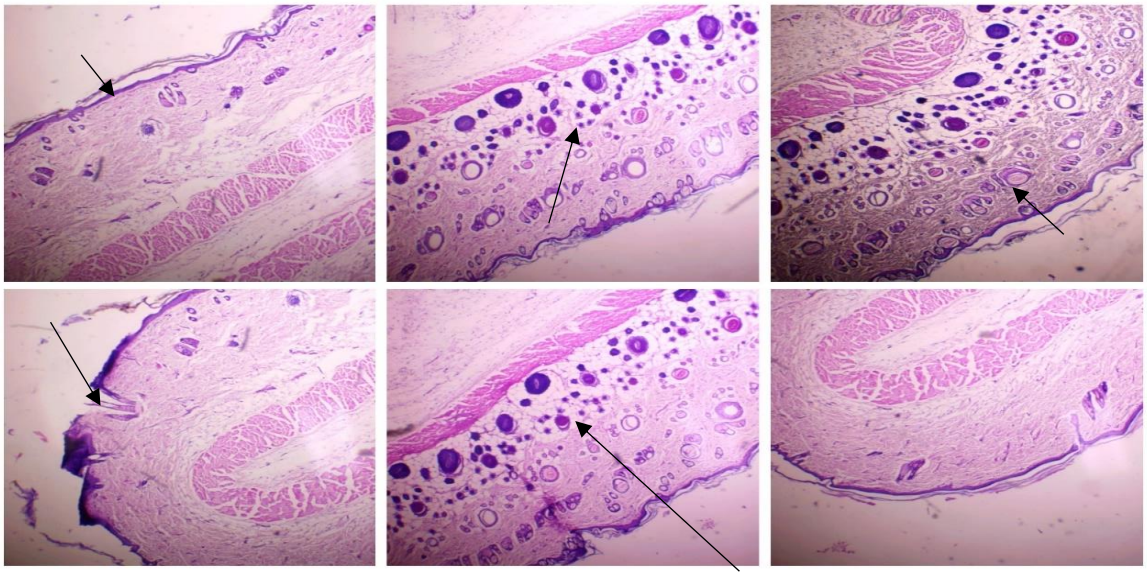

Figure 20: Microscopic images of a piece of dry epidermal skin (Composition 2)

In treated dry skin samples, a gradual restoration of epidermal regularity was observed, with a marked improvement in skin cell cohesion and a relative increase in tissue hydration. Collagen

fibers also appeared more regular and cohesive, with a marked decrease in inflammatory markers.

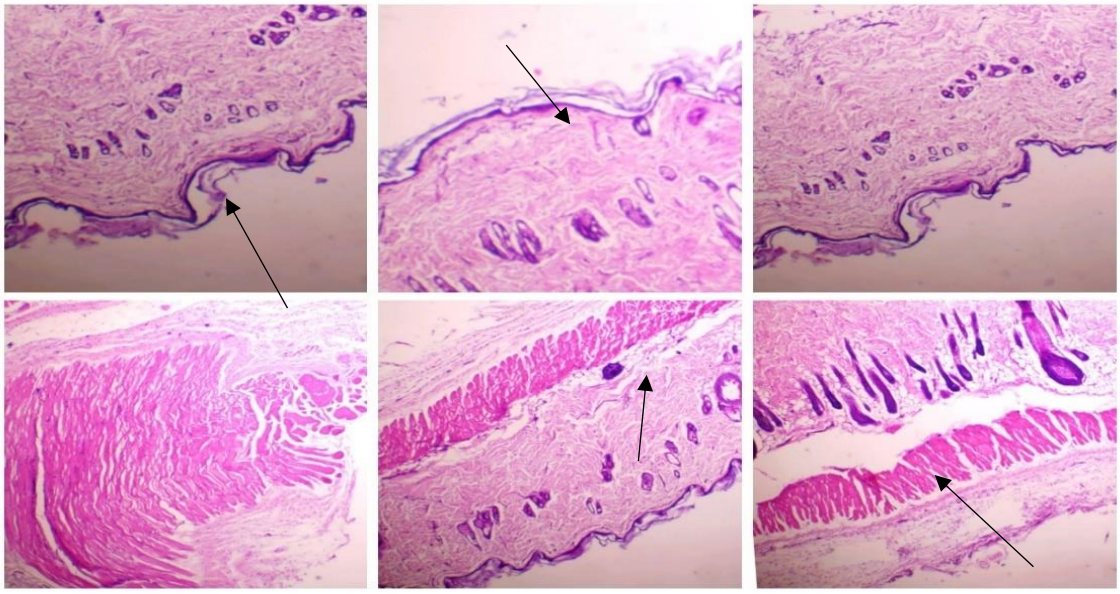

Figure 21: Microscopic images of a skin sample treated with spirulina serum for oily skin x40 (HA)

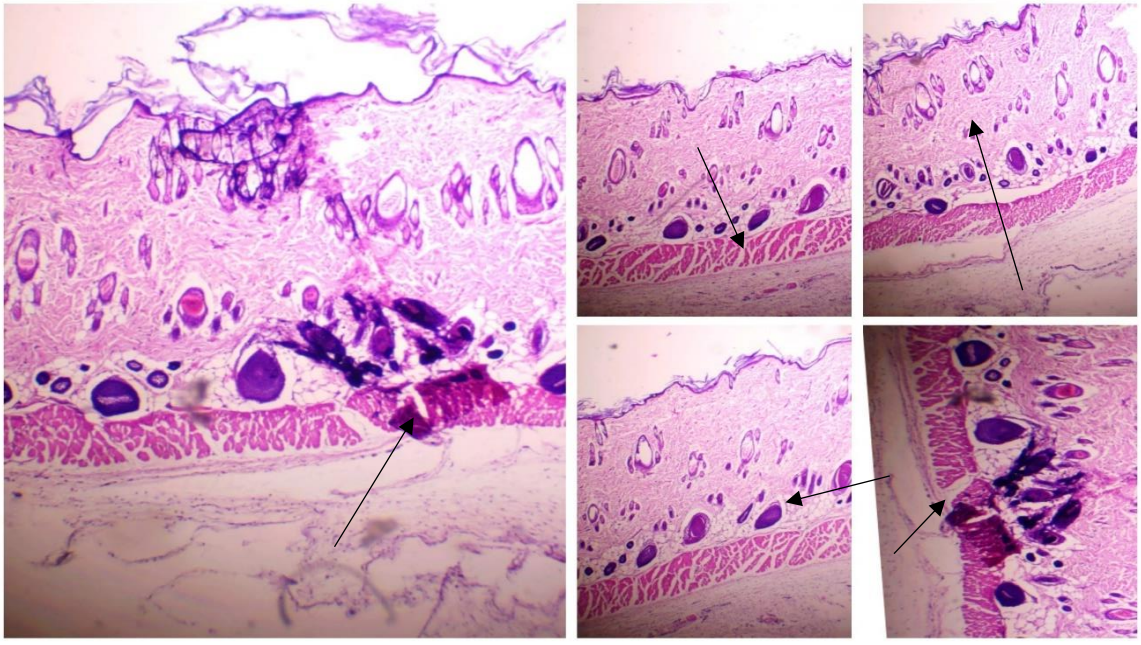

Figure 22: Microscopic images of a skin sample treated with spirulina serum for oily skin

In treated oily skin samples, a decrease in sebaceous gland hyperactivity was observed compared to the pathological condition, along with a reduction in sebum accumulation within the tissues. Histological sections also showed improved epidermal layer regularity and a decrease in inflammatory infiltration, reflecting a partial restoration of the skin's tissue balance.

Previous studies have shown that spirulina extracts possess a clear ability to reduce oxidative stress (ROS) and decrease skin inflammation, thus contributing to the protection of fibroblasts and keratinocytes and improving the skin's tissue environment. Other research has demonstrated that spirulina promotes cell regeneration and increases fibroblast proliferation, along with improved collagen deposition within the dermis, which directly impacts the speed of skin healing and improves its tissue structure.

In animal studies, topical application of spirulina extract has been observed to accelerate wound healing, reduce inflammatory infiltration, and enhance connective tissue remodeling while improving collagen fiber organization and reducing scar tissue formation.

Therefore, the improvements observed in the dry and oily skin samples treated in your study—such as epidermal smoothing, reduced inflammation, and improved collagen fibers—are highly consistent with published findings, confirming that spirulina serum has a positive histological effect, namely:

*Promoting skin cell renewal

* Reducing localized inflammation

* Supporting collagen fiber remodeling

mproving the skin's tissue balance

III-6 Bacteriological Analysis

Table07 Results of dry formule products

determinination	Results
Germes aerobic	50gr/l
Escherchia coli	Abs
Pseudomonas aeruginosa	Abs
Staphylococcus aureus	Abs
Yeasts &fungi	Abs

Table08 Results of oily formule product

determinination	Results
Germes aerobic	Abs
Escherchia coli	Abs
Pseudomonas aeruginosa	Abs
Staphylococcus aureus	Abs
Yeasts &fungi	Abs

The bacteriological analysis of the final serum formulation showed the absence of any visible bacterial growth on the culture media after the incubation period. No bacterial colonies were detected compared to the control, indicating that the prepared serum was microbiologically stable and free from bacterial contamination.

This result may be explained by the antimicrobial properties of Spirulina extract, which contains several bioactive compounds such as phenolic compounds, peptides, fatty acids, and phycocyanin known for their inhibitory effects against different bacterial strains. In addition, the suitable pH of the formulation and the hygienic conditions maintained during the preparation process may have contributed to preventing bacterial proliferation.

The absence of bacterial contamination also reflects the good quality of the raw materials and the effectiveness of the preservation conditions used during formulation and storage. Similar findings were reported in previous studies demonstrating that cosmetic products enriched with natural bioactive compounds can exhibit significant microbiological stability and safety.

Therefore, the obtained results confirm that the prepared Spirulina serum is microbiologically safe and suitable for topical application without risk of bacterial contamination.

General conclusion

Conclusion

In conclusion, this study, which focused on the formulation and evaluation of a natural facial serum for skin care, made it possible to obtain several important findings reflecting the quality and potential effectiveness of the prepared product. The results showed that the serum possessed a pH ranging from 5.06 to 5.25, values that are compatible with the physiological pH of the skin (4.5–5.5), indicating that the formulation is suitable for topical application without disturbing the natural skin barrier or causing irritation. The physicochemical evaluation also demonstrated good homogeneity and acceptable stability of the serum, with no noticeable phase separation or undesirable changes in color and odor during the storage period, confirming the success of the formulation process. Moreover, microbiological analyses revealed the absence of bacterial contamination in the final product, which highlights the effectiveness of the sterilization and preservation conditions used during preparation and ensures the microbiological safety of the serum. Preliminary observations also suggested an improvement in skin hydration and softness in treated samples compared with untreated controls, which may be attributed to the presence of natural bioactive compounds with moisturizing and antioxidant properties. The significance of this study lies in its contribution to the development of safer and more natural cosmetic products as alternatives to synthetic formulations, while also providing a scientific basis for understanding the effects of natural serums on skin quality through physicochemical and microbiological evaluations. However, the study presents certain limitations, including the small sample size, the absence of large-scale clinical testing, and the relatively short study duration, which did not allow the evaluation of long-term stability and prolonged effects on the skin. In addition, no advanced cellular or histological investigations were performed to better explain the mechanisms of action of the active ingredients. Therefore, further studies are recommended, including extensive clinical trials involving larger populations and longer treatment periods, stability studies under different environmental conditions, and in vitro cellular analyses to better characterize the biological effects of the formulation. Future perspectives may also include improving the serum composition by incorporating additional natural active ingredients such as vitamins and plant antioxidants, comparing the product with commercial cosmetic formulations, and developing other pharmaceutical or cosmetic forms such as creams and gels. Overall, this work represents a promising preliminary step toward the development of effective, safe, and natural skin-care products with potential applications in the cosmetic field.

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Annex

Hematological and Renal Biochemical Analyses

I. HEMATOLOGICAL ANALYSES

1. Principle

Hematological parameters are determined using an automated hematology analyzer based on the impedance or flow cytometry principle. Blood cells suspended in an electrolyte solution pass through an aperture. Changes in electrical impedance are proportional to cell size, allowing counting and differentiation of cells.

2. Parameters Measured

- Red blood cells (RBC)
- White blood cells (WBC)
- Hemoglobin concentration (Hb)
- Hematocrit (HCT)
- Platelets (PLT)

3. Sample

Whole blood collected in EDTA tubes.

4. Procedure

Samples are gently mixed and analyzed using the hematology analyzer according to the manufacturer's instructions.

II. RENAL BIOCHEMICAL ANALYSES

Serum is separated by centrifugation of clotted blood at 3000 rpm for 10 minutes. Renal function is assessed by the following parameters.

A. Urea

1. Principle

Urea is hydrolyzed by urease to produce ammonia and carbon dioxide. The released ammonia reacts with hypochlorite and salicylate in an alkaline medium to form indophenol blue. The intensity of the color measured at 578 nm is proportional to the urea concentration.

2. Reaction

$$\text{Urea} + H_2O + \text{urease} \rightarrow 2NH_3 + CO_2$$

$$NH_3 + \text{Hypochlorite} + \text{Salicylate} \rightarrow \text{Indophenol blue (colored compound)}$$

Reagent	Composition	Concentration
R1	Urease Buffer (pH 7.0)	≥ 10000 U/L
R2	Sodium salicylate Sodium hypochlorite Sodium nitroprusside	20 mmol/L 10 mmol/L 0.5 mmol/L
Standard	Urea	50 mg/dL

B. Creatinine

1. Principle

Creatinine reacts with picrate in an alkaline medium (Jaffé reaction) to form an orange-red colored complex. The absorbance measured at 520 nm is proportional to the creatinine concentration.

2. Reaction

$$\text{Creatinine} + \text{Picrate (alkaline)} \rightarrow \text{Orange-red complex}$$

C. Uric Acid

1. Principle

Uric acid is oxidized by uricase to allantoin and hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine (4-AAP) and phenol in the presence of peroxidase (POD) to form a red quinoneimine dye. The intensity of the color measured at 520 nm is proportional to the uric acid concentration.

2. Reaction

$$\text{Uric acid} + H_2O + \text{uricase} \rightarrow \text{Allantoin} + H_2O_2$$

$$H_2O_2 + \text{4-AAP} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine (red dye)}$$

Reagent	Composition
R1	Buffer (pH 8.0) Uricase Peroxidase
R2	4-Aminoantipyrine Phenol
Standard	Uric acid (6 mg/dL)

D. General Procedure (Biochemical Assays)

- Bring reagents and samples to room temperature.
- Pipette into cuvettes:
  - Blank: Reagent
  - Standard: Reagent + Standard
  - Sample: Reagent + Sample
- Mix and incubate as specified for each assay.
- Read the absorbance against the blank at the appropriate wavelength.
- Calculate the concentration using the standard or calibration curve.

الشهابي لتحاليل مراقبة النوعية و المطابقة

Analyses de Contrôle de Qualité et de Conformité
Analyses Physico - Chimique et Microbiologique
Autorisation Ministérielle N°: 082 du 04/03/2007
Expert assermenté inscrit au cour - خبير محلف مسجل لدى المجلس القضائي
رقم التعريف المهني: 062020002855

Bulletin d'Analyses

Microbiologie — Jou mois: — Année:

N°: Y 00035 /2026 — Établi le: 26 5 26

Code Client: 0 — Nom du Client: طلحة اخلاص بوعقلة أسماء

Genre d'Analyse: 2 — Adresse Client: المغير

Produit: زيت خاص بالشرة الذهبية

Prélèvement du 20 5 2026 — par: Le Client

Echant. reçu le 20 5 2026 — Sous le N°: V 00094

Lancé le 20 5 2026

Détermination	Résultats 1er Ech	Normes Arrêtée du 21/10/2019 JO N° 16 du 24/03/2020 m	M	Methodes
Germes aérobies à 30°C	Abs	$\leq 10^2$ gr/g	$\leq 2 \times 10^3$ gr/g	NA ISO 21149 (NA 8287)
Levures et Moisissures à 25°C	Abs	≤100 gr/g		NA ISO 16212
Escherchia Coli à 44 °C	Abs	Absence dans 1 g ou 1 ml		NA ISO 21150 (NA14808)
Pseudomonas aeruginosa à 22°C	Abs	Absence dans 1 g ou 1 ml		NA ISO 22717
Staphylococus aureus à 37°C	Abs	Absence dans 1 g ou 1 ml		NA ISO 22718 (NA 14809)

Interprétation: Echantillon de **Qualité Bactériologique Satisfaisante** aux Normes de l'Arrêtée Interministériel du 21/10/2019 JO N° 16 du 24/03/2020 /

NB: Ce bulletin est identique à la souche archivée chez le laboratoire qui ne contient aucun surcharge ou correction et dans le cas contraire la présente feuille sera annulée./

Le Laborantin

El Oued le: 26 Mai 2026
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الأشهب معاوية
خبير محلف مسجل لدى المجلس القضائي

الدكتورة حموية أميمة
بيوكيمياء تطبيقية

Dossier enregistré sous: 70521052041018810202619

الشهابي لتحاليل مراقبة النوعية و المطابقة

Analyses de Contrôle de Qualité et de Conformité
Analyses Physico - Chimique et Microbiologique
Autorisation Ministérielle N°: 082 du 04/03/2007
Expert assermenté inscrit au cour - خبير محلف مسجل لدى المجلس القضائي
رقم التعريف المهني: 062020002855

Bulletin d'Analyses

Microbiologie

N°: Y 00036 /2026 — Jou mois: — Année: — Etabli le: 26 5 26

Code Client: 0 — Nom du Client: ملحة اخلاص جوهة أسماء

Genre d'Analyse: 2 — Adresse Client: المغير

Produit: زيت خاص بالبشرة الجافة

Prélèvement du: 20 5 2026 — par: Le Client

Echant. reçu le: 20 5 2026 — Sous le N°: V 00095

Lancé le: 20 5 2026

Détermination	Résultats 1° Ech	Normes Arrêtée du 21/10/2019 JO N° 16 du 24/03/2020 m	M	Méthodes
Germes aérobies à 30°C	50 gr/g	$\leq 10^3$ gr/g	$\leq 2 \times 10^3$ gr/g	NA ISO 21149 (NA 8287)
Levures et Moisissures à 25°C	Abs	≤ 100 gr/g		NA ISO 16212
Escherchia Coli à 44 °C	Abs	Absence dans 1 g ou 1 ml		NA ISO 21150 (NA14808)
Pseudomonas aeruginosa à 22°C	Abs	Absence dans 1 g ou 1 ml		NA ISO 22717
Staphylococus aureus à 37°C	Abs	Absence dans 1 g ou 1 ml		NA ISO 22718 (NA 14809)

Interprétation: Suivant les paramètres recherchés, Présence des Germes aérobies à 30°C mais à un nombre ne dépassant pas les limites des Normes de l'Arrêtée Interministériel du 21/10/2019 JO N° 16 du 24/03/2020.Echantillon de **Qualité Bactériologique Satisfaisante** /

NB: Ce bulletin est identique à la souche archivée chez le laboratoire qui ne contient aucun surcharge ou correction et dans le cas contraire la présente feuille sera annulée./

El Oued le: 26 Mai 2026

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