World Journal of Pharmaceutical Science and Research

www.wjpsronline.com

Research Article

ISSN: 2583-6579
SJIF Impact Factor: 5.111
Year - 2024
Volume: 3; Issue: 5
Page: 01-16

FORMULATION AND EVALUATION OF SPIRULINA IN HERBAL MOISTURIZING CREAM WITH ANTI MICROBIAL ACTIVITY

N. Jayaramakani*[1], K. Saifullaha[2], S. Baladhandapani[2], L. Kumaraguru[2], K. Yuvaraj[2] and R. Ranjith[2]

[1]Head of the Department, Faculty of Pharmacy, Bharath Institute of Higher Education and Research, Chennai 600073, India.
[2]Final Year B.Pharm, Faculty of Pharmacy, Bharath Institute of Higher Education and Research, Chennai 600073, India.

Article Received: 22 July 2024 | | Article Revised: 13 August 2024 | | Article Accepted: 04 September 2024

***Corresponding Author: N. Jayaramakani**
Head of the Department, Faculty of Pharmacy, Bharath Institute of Higher Education and Research, Chennai 600073, India.
DOI: https://doi.org/10.5281/zenodo.13868742

How to cite this Article: N. Jayaramakani, K. Saifullaha, S. Baladhandapani, L. Kumaraguru, K. Yuvaraj and R. Ranjith (2024). FORMULATION AND EVALUATION OF SPIRULINA IN HERBAL MOISTURIZING CREAM WITH ANTI MICROBIAL ACTIVITY. World Journal of Pharmaceutical Science and Research, 3(5), 01-16. https://doi.org/10.5281/zenodo.13868742

ABSTRACT

Spirulina, a nutrient-rich blue-green algae, is incorporated as the active ingredient in this formulation due to its high content of vitamins, minerals, and antioxidants, all of which are beneficial for skin health. The formulation includes a carefully selected combination of emollients, humectants, emulsifiers, and preservatives to create a stable and effective cream. Key ingredients include shea butter, coconut oil, glycerin, emulsifying wax, and aloe vera gel. The formulation process involves heating and combining the oil and water phases, followed by cooling and the addition of spirulina and other heat-sensitive components. The resulting cream is then evaluated for its physicochemical properties, such as appearance, pH, viscosity, and spreadability. Stability tests, including accelerated stability testing at various temperatures and freeze-thaw cycles, are conducted to ensure the product's durability. The moisturizing efficacy of the cream is measured through in vivo testing to determine its ability to enhance skin hydration. The cream may also help reduce skin sensitivity, improve skin tone and texture, and conceal imperfections. Spirulina is rich in bioactive compounds, including phycocyanin, chlorophyll, proteins, vitamins (B1, B2, B3, B6, B9, C, E), minerals (iron, magnesium, calcium, potassium), and essential fatty acids. These components contribute to its antioxidant, anti-inflammatory, and immune-boosting properties. Spirulina works by providing essential nutrients, reducing oxidative stress, enhancing immune function, and supporting overall health. Herbal medications or extracts are commonly used in topical formulations and cosmetics. For example, Arctium lappa L. (Asteraceae) has been used to treat inflammatory conditions and various skin issues, making it a suitable herbal product for healing dry skin. The cream contains several natural ingredients, including liquid paraffin, vitamin E, jasmine oil, Aloe barbadensis (aloe vera leaves), Azadirachta indica (neem), beeswax, and rose oil. The selection of ingredients is based on their therapeutic properties and their ability to enhance the cream's effectiveness. Spirulina, known as a "superfood" due to its nutrient density, is high in protein, iron, antioxidants, and omega-3 and omega-6 fatty acids, which support heart health, boost energy, reduce inflammation, and improve overall well-being. Spirulina is commonly consumed as a supplement in pill or powder form and can also be added to smoothies, salads, or other recipes. The antioxidant and anti-inflammatory components of the herbal cream formulation help reduce oxidative stress and inflammation. Additionally, its high vitamin E content hydrates and nourishes the skin, preventing it from drying out.

KEYWORDS: Aloe vera gel, Arthospira platensis, moisturizing cream, Antioxidant, Acne vulgaris.

INTRODUCTION

Spirulina, a single-cell protein, is a rich source of essential nutrients and vitamins, making it a valuable component in nutritious food production. This cyanobacterium, including spirulina, holds potential medicinal applications. Studies suggest that combining spirulina with probiotics might enhance healthy gut flora development, although human trials primarily use spirulina supplements. The genus Spirulina, part of the Oscillatoriaceae family, utilizes dissolved carbon dioxide in saltwater for reproduction and thrives in hot, humid climates with abundant sunlight due to its photosynthetic nature.

Spirulina species, particularly Arthrospira platensis, are high in protein (approximately 60-70%) and contain beneficial compounds such as phycocyanin, beta-carotene, B vitamins (B12, A, E, D), various minerals (e.g., Ca, Fe, P, I, Mg, Zn, Se, Cu, Mn, Cr, K, Na), unsaturated fatty acids (e.g., gamma-linolenic acid), and the enzyme superoxide dismutase (SOD). Phycocyanin, a blue pigment composed of α and β polypeptide subunits, is notable for its health benefits. Spirulina contains potent antioxidants such as phycocyanin, chlorophyll, and beta-carotene.

When formulating topical products with spirulina, factors like dermal toxicity, component compatibility, and skin penetration efficiency are crucial. Spirulina production involves cultivation, harvesting, and processing, typically using raceway ponds with paddle wheels for agitation. Major spirulina-producing countries include the United States, Thailand, India, Taiwan, China, Pakistan, and Burma. Spirulina's rich nutrient profile makes it an excellent dietary supplement and beneficial for treating conditions like acne vulgaris, a chronic inflammatory skin disease affecting adolescents due to increased sebum production, follicle hyperkeratinization, bacterial colonization, and inflammation. Spirulina's applications extend to cosmetics and biomedicine, including anti-aging creams, emollients, and wound healing patches. It also possesses antiviral, antibacterial, antifungal, anti-inflammatory, and anticancer properties.

Discovered by Spanish scientist Hernando Cortez in 1519, spirulina's health benefits were later highlighted by Pierre Dangeard and botanist Jean Leonard. Commercial spirulina production began in 1969 with the French establishment of the Sosa Texcoco facility. Spirulina can be found in health food stores and is sold mainly as a dietary supplement in the form of health drinks or tablets. Microalgae have been used for more than 10 years as dietary supplements without significant side effects.

In recent years, the skincare industry has witnessed a significant shift towards natural ingredients, driven by consumer demand for safer and more sustainable products. Among these ingredients, Spirulina, a blue-green microalgae, has emerged as a promising candidate due to its rich nutritional profile and diverse health benefits. This exploration focuses on the formulation and evaluation of Spirulina in moisturizing cream, aiming to harness its potential for enhancing skin health and hydration. Spirulina, scientifically classified as Arthrospira platensis or Arthrospira maxima, belongs to the Cyanobacteria phylum. It thrives in alkaline water bodies, predominantly in tropical and subtropical regions. Historically, Spirulina has been consumed as a dietary supplement and food source by various civilizations, including the Aztecs and inhabitants of Chad.

The skincare industry's evolution has emphasized the importance of moisturizing products in maintaining skin health. Moisturizers are essential for preventing dryness, improving skin texture, and protecting against environmental stressors. With increasing awareness of the potential risks associated with synthetic chemicals in cosmetics, there is a growing preference for natural ingredients known for their safety and efficacy. Spirulina's bioactive compounds,

coupled with its moisturizing and antioxidant properties, make it a compelling choice for skincare formulations aimed at enhancing hydration, reducing signs of aging, and promoting overall skin wellness.

Biochemical Composition of Spirulina

Spirulina's exceptional biochemical composition underpins its potential for skincare applications. Rich in proteins, vitamins, minerals, essential fatty acids, and antioxidants, Spirulina offers numerous benefits for skin health:

- **Proteins:** The high-quality protein content in Spirulina supports collagen synthesis, essential for maintaining skin elasticity and firmness. It also aids in repairing and rejuvenating skin cells, contributing to a smoother and more youthful appearance.
- **Vitamins and Minerals:** Essential vitamins such as B complex and antioxidants like vitamins C and E protect the skin from oxidative stress, UV damage, and premature aging. Minerals like zinc and selenium promote skin regeneration and help maintain a healthy skin barrier.
- **Essential Fatty Acids:** Gamma-linolenic acid (GLA) and omega-3 fatty acids in Spirulina moisturize the skin, enhance its lipid barrier function, and reduce inflammation, helping to soothe dry, irritated skin conditions such as eczema and dermatitis.
- **Antioxidants:** Phycocyanin, chlorophyll, and beta-carotene in Spirulina exhibit potent antioxidant properties, neutralizing free radicals that cause cellular damage and accelerate aging processes. These antioxidants also contribute to a brighter complexion and improved skin tone.

Benefits and Application

Incorporating Spirulina into herbal moisturizing creams provides more than just hydration and antioxidant protection. Its nutrient-rich composition supports skin health by facilitating cellular repair, stimulating collagen production, and promoting overall skin rejuvenation. Regular application of Spirulina-based creams can improve skin tone, reduce the visibility of fine lines and wrinkles, and enhance the skin's innate radiance. The natural origins of Spirulina and other herbal ingredients resonate with environmentally conscious consumers seeking sustainable skincare solutions that emphasize both effectiveness and environmental stewardship.

MATERIAL AND METHODS

SPIRULINA

Spirulina, or Arthrospira, a type of blue-green algae, gained prominence when NASA successfully used it as a nutritional supplement for astronauts on space missions. It can modulate immune responses and demonstrates anti-inflammatory properties by inhibiting the release of histamine from mast cells. Spirulina platensis contains functional bioactive components, such as phenolic phytochemicals, which exhibit antibacterial activity against various pathogenic bacteria and fungi. With its ability to block mast cells from producing histamine, it possesses antiinflammatory effects and regulates immune function. Spirulina consists of 55-70% protein, 1525% polysaccharides, 56% total fat, 6-13% nucleic acids, and 2.2-4.8% minerals. Structurally, the 25% branching polysaccharide in A. platensis is similar to glycogen.

Fig. 1: Spirulina (Arthospira platensis).

Synonyms: Arthrospira maxima, Arthrospira platensis, Spirulina fusiformis, Spirulina maxima, Spirulina pacifica, Spirulina platensis.

Biological Source: Spirulina is the dried biomass of the oxygenic photosynthetic bacteria Arthrospira platensis, found in both fresh and marine waters globally.

Geographical Source: The species A. platensis is primarily found in Africa but also exists in Asia. A. maxima is believed to be found in Mexico and California. Despite historical reasons, the term “spirulina” remains in use.

Chemical Constituents: Spirulina contains fatty acids like linoleic acid, docosahexaenoic acid, eicosapentaenoic acid, arachidonic acid, and stearidonic acid.

Nutritional Profile: Spirulina is recognized for its rich nutrient content. Per 100 grams, it provides approximately:

- Calories: 290 kcal
- Protein: 57.47 grams

Total Fat: 7.72 grams, including:

- Saturated Fat: 2.65 grams
- Monounsaturated Fat: 0.675 grams
- Polyunsaturated Fat: 2.08 grams

Carbohydrates: 23.9 grams, including:

- Dietary Fiber: 3.6 grams
- Sugars: 3.1 grams

Vitamins:

- Vitamin A (as β-carotene): 5900 IU
- Vitamin B12: 44.8 mcg
- Vitamin E: 5 mg
- Vitamin K1: 25.5 mcg

Minerals:

- Iron: 28.5 mg
- Calcium: 120 mg
- Magnesium: 195 mg
- Potassium: 1363 mg
- Phosphorus: 118 mg

Essential Fatty Acids: γ-Linolenic Acid (GLA): 0.44 grams.

Amino Acids: Spirulina provides a full spectrum of essential amino acids and many non-essential ones, such as Isoleucine, Leucine, Lysine, Threonine, Tryptophan, Valine, Methionine, Phenylalanine, Arginine, Aspartic acid, Cysteine, Glutamic acid, Glycine, Histidine, Proline, Serine, and Tyrosine.

This nutrient-dense profile makes Spirulina a highly valued dietary supplement, especially noted for its high protein content, essential fatty acids, and abundance of vitamins (notably B12 and βcarotene) and minerals (such as iron and potassium).

USES

Dietary Supplement: Spirulina serves as a powerful dietary supplement by offering a rich array of proteins, vitamins, and minerals. It boosts energy and stamina, enhancing physical performance and reducing fatigue.

Medical Applications: In medical contexts, spirulina acts as a potent antioxidant, mitigating oxidative stress and protecting cells from damage. It also possesses anti-inflammatory properties, which can help alleviate conditions such as arthritis, and supports immune system function by enhancing the body's defense mechanisms.

Cosmetics and Skincare: For skincare, spirulina's antioxidant qualities help shield the skin from damage and promote a youthful appearance. It also provides hydration and essential nutrients, making it beneficial for moisturizing the skin.

Health Benefits: Spirulina is highly nutritious, making it an excellent supplement. It supports the body's detoxification processes, particularly in removing heavy metals. Additionally, it may contribute to lowering bad cholesterol (LDL) and increasing good cholesterol (HDL), and helps stabilize blood sugar levels, which can be advantageous for those with diabetes.

Potential Risks: Possible risks include allergic reactions, such as skin rashes or gastrointestinal issues. Spirulina from contaminated sources may contain harmful toxins like microcystins. It can also potentially overstimulate the immune system, which might worsen autoimmune conditions.

Therapeutic Uses

Treatment of Acne Vulgaris: Spirulina can impact acne vulgaris, a chronic inflammatory skin condition affecting adolescents. It influences this condition by increasing sebum production, causing follicular hyperkeratinization, promoting bacterial growth, and triggering inflammation. Spirulina's role extends to various cosmetic and biomedical uses:

1. **Anti-aging Creams:** It reduces wrinkles and fine lines due to its antioxidant properties.

2. **Refreshing and Regenerating Products:** These products help rejuvenate the skin for a youthful appearance.
3. **Emollients and Anti-irritants:** Used in skin treatments to soothe and soften the skin.
4. **Wound Healing Patches:** Its bioactive compounds aid in healing and reducing inflammation in damaged skin.

Additional Properties: Spirulina platensis is known for its high vitamin B12 and amino acid content. It is recognized for its antiviral, anticancer, cholesterol-lowering, blood sugar-regulating, anti-inflammatory, anti-metastatic, and antioxidant properties.

Anti-inflammatory Activity: Anti-inflammatory creams often include corticosteroids, NSAIDs, and natural extracts like aloe vera and jasmine. These ingredients work by inhibiting proinflammatory mediators and reducing the immune response, helping to alleviate symptoms of eczema, psoriasis, and dermatitis. Regular application, as directed, can help restore the skin's barrier function and reduce irritation and redness.

Table No.1.

Ingredient	Role
Spirulina Powder	Rich in antioxidants, vitamins, and minerals; provides anti-aging and nourishing properties.
Vitamin E Oil	Antioxidant; helps protect skin cells, moisturizes, and acts as a preservative.
Aloe Vera Gel	Soothing and hydrating; helps to calm and moisturize the skin.
Neem Oil	Antibacterial and antifungal properties; helps to treat and prevent skin infections.
Coconut Oil	Deeply moisturizing and nourishing; helps to soften and hydrate the skin.
Shea Butter	Deeply moisturizing; helps to nourish and protect the skin barrier.
Methyl Paraben	Preservative; prevents microbial growth and extends shelf life.
Jasmine Oil	Imparts a pleasant fragrance and provides soothing and moisturizing benefits.
Rose Oil	Adds a luxurious fragrance and has anti- inflammatory and moisturizing properties.
Liquid Paraffin	Acts as a moisturizer; leaves a protective layer on the skin to prevent moisture loss.
Paraffin Wax	Enhances the cream's texture and provides a protective barrier on the skin.
Ceto stearyl Alcohol	Emulsifier and thickener; helps to blend oil and water phases, giving the cream a smooth texture.
Cetyl Alcohol	Emulsifier and emollient; helps to soften and soothe the skin, providing a smooth texture.
Lemon grass oil	It is a Cleaning agent. It protects against bugs and insects. Potentially improve gut health. Improve skin and hair.

PREPARATION AND FORMULATION

To create five different formulations for moisturizing creams, you will need to carefully prepare both the oil phase and aqueous phase at specific temperatures to ensure a smooth emulsion. Here's a step-by-step process for the formulation:

1. **Oil Phase Preparation**

- **Ingredients**: Cetostearyl alcohol, paraffin wax, liquid paraffin, cetyl alcohol.
- **Procedure**: Melt the oil phase ingredients together by heating them to 70°C. Ensure that all the components are thoroughly melted and well mixed to form a homogeneous mixture.

2. **Aqueous Phase Preparation**

- **Ingredients**: Water, preservatives (like methyl paraben), spirulina extract.
- **Procedure**: Simultaneously, heat the aqueous phase ingredients to 70°C. Ensure that the water and preservatives are well mixed, and the spirulina extract is fully dissolved and uniformly dispersed in the solution.

3. **Combining Phases**

- Slowly add the oil phase into the aqueous phase while continuously stirring. This process should be done gradually to form a stable emulsion.

- Continue stirring until the mixture cools down to around 40°C. This cooling phase is crucial for the emulsion to stabilize and thicken properly.

4. **Addition of Active Ingredients and Essential Oils**

- Once the emulsion has cooled to around 40°C, incorporate the active ingredients and essential oils as per each formulation's specific requirements. For example, you can add vitamin E oil for its antioxidant properties, neem oil for its antibacterial benefits, and essential oils such as lavender, lemongrass, jasmine, or rose for fragrance and additional skin benefits.

5. **Final Mixing and Packaging**

- Ensure thorough mixing to achieve a homogeneous cream.
- Transfer the finished cream into sterilized containers to maintain its quality and prevent contamination.

Formulation 1

Oil Phase

- Cetostearyl alcohol
- Paraffin wax
- Liquid paraffin
- Cetyl alcohol

Aqueous Phase

- Water
- Methyl paraben
- Spirulina powder

Additional Ingredients

- Jasmine oil
- Coconut oil
- Neem oil
- Aloe vera gel
- Vitamin E oil

Procedure

1. Melt the oil phase ingredients at 70°C.
2. Heat the aqueous phase ingredients to 70°C.
3. Slowly add the oil phase to the aqueous phase with continuous stirring.
4. Cool the mixture to 40°C while stirring.
5. Add jasmine oil, coconut oil, neem oil, aloe vera gel, and vitamin E oil, then mix thoroughly.
6. Transfer to sterilized containers.

Formulation 2

Oil Phase

- Cetostearyl alcohol
- Paraffin wax
- Liquid paraffin
- Cetyl alcohol

Aqueous Phase

- Water
- Methyl paraben
- Spirulina powder

Additional Ingredients

- Shea butter
- Neem oil
- Aloe vera gel
- Vitamin E oil
- Lemon grass oil

Procedure

1. Melt the oil phase ingredients at 70°C.
2. Heat the aqueous phase ingredients to 70°C.
3. Slowly add the oil phase to the aqueous phase with continuous stirring.
4. Cool the mixture to 40°C while stirring.
5. Add shea butter, neem oil, aloe vera gel, vitamin E oil and Lemon grass inoil then mix thoroughly.
6. Transfer to sterilized containers.

Formulation 3

Oil Phase

- Cetostearyl alcohol
- Paraffin wax
- Liquid paraffin
- Cetyl alcohol

Aqueous Phase

- Water
- Methyl paraben
- Spirulina powder

Additional Ingredients

- Neem oil
- Aloe vera gel

- Vitamin E oil
- Rose oil

Procedure

1. Melt the oil phase ingredients at 70°C.
2. Heat the aqueous phase ingredients to 70°C.
3. Slowly add the oil phase to the aqueous phase with continuous stirring.
4. Cool the mixture to 40°C while stirring.
5. Add neem oil, aloe vera gel, vitamin E oil, and rose oil, then mix thoroughly.
6. Transfer to sterilized containers.

INGREDIENTS AND QUANTITY

Table No. 2.

Ingredient	Formulation 1	Formulation 2	Formulation 3
Oil Phase			
Cetostearyl alcohol	5 g	5 g	5 g
Paraffin wax	5 g	5 g	5 g
Liquid paraffin	10 g	10 g	10 g
Cetyl alcohol	5 g	5 g	5 g
Aqueous Phase			
Water	60 g	60 g	60 g
Methyl paraben	1 g	1 g	1 g
Spirulina powder	2 g	2 g	2 g
Additional Ingredients			
Jasmine oil	0.5 g		
Coconut oil	5 g		
Neem oil	2 g	2 g	2 g
Aloe vera gel	5 g	5 g	5 g
Vitamin E oil	1 g	1 g	1 g
Shea butter		5 g	
Rose oil			0.5 g
Lemon grass oil		0.5 g	

EVALUATION PARAMETERS OF MOISTURIZING CREAM

pH TEST

This test evaluates whether the product is acidic or alkaline. Creams with a pH similar to that of the skin (about pH 4.5 to 6.5) are considered more suitable and less likely to cause irritation. The pH is determined using pH strips or a pH meter. Adjusting the pH can help maximize the cream's efficacy while reducing the likelihood of skin reactions. **Apparatus or equipment used**: pH meter

Table No. 3.

Trial	pH
1	6.2
2	6.1
3	6.2
Average	6.2 ± 0.06

The pH of the spirulina cream is **6.2 ± 0.06.**

Marketed sample: pH of the cream was found to be range is 6.5 – 7.2. The herbal formulation was shown pH nearer to skin is required pH 7.

DIFFUSION TEST

The Franz diffusion cell is a widely used in vitro method for evaluating the diffusion and permeation characteristics of topical formulations, including moisturizing creams. In this test, the moisturizing cream is applied to the donor compartment, which is separated from the receptor compartment by a synthetic membrane or excised skin. The receptor compartment is filled with a suitable medium that mimics the physiological conditions of the skin. Over a specified period, samples are collected from the receptor compartment to measure the amount of active ingredient that has diffused through the membrane. This diffusion test setup allows for precise control and measurement of diffusion rates, providing valuable data on the cream's efficacy in delivering active ingredients into the skin. The results from a Franz diffusion cell diffusion test can help in optimizing formulation properties and ensuring consistent performance of the moisturizing cream.

Apparatus/Equipment used: Franz Diffusion Cell and UV Spectrophotometer

Media used: Phosphate buffer pH 7.4

Time of study: 4 hours

RPM: 100 RPM

Results: max of the sample 255 nm

Dilution factor: 0.5

Time in minutes	Absorbance in nm*
10	0.0224±0.0005
30	0.0479±0.0074
60	0.0658±0.0029
90	0.0722±0.0033
120	0.0798±0.0017
180	0.0952±0.0016
240	0.1917±0.0116

n=3, mean=±3

A standard 25mm Franz Cell also has a receptor volume of 20ml but the cell should not be referred to as a 20ml Franz Cell it is a 25mm Franz Cell with a 20ml receptor volume. The outer diameter of the joint on a 25mm Franz Cell is about 42mm, but the cell is properly referred to as a 25mm Franz Cell.

Acid Value

This test determines the amount of free fatty acids in the product, indicating the level of hydrolytic rancidity and overall quality. It involves titrating the cream with a known concentration of a base (typically potassium hydroxide) to neutralize the free fatty acids and calculating the amount of base needed. A lower acid value implies more stability and quality, while higher levels indicate fat and oil deterioration, which can compromise the cream’s safety, efficacy, and shelf life.

Saponification Value

This test determines the quantity of alkali necessary to saponify the fats and oils in the product, providing information about the average molecular weight of the fatty acids present. It involves titrating the mixture after heating it with alcoholic potassium hydroxide.

UV ANALYSIS OF DIFFUSION STUDY SAMPLES

Table No. 4.

S. No	Time (in Minutes)	Absorbance	
		Batch A	Batch B
1	10	0.0227	0.0220
2	30	0.0531	0.0427
3	60	0.0678	0.0637
4	90	0.0698	0.0745
5	120	0.0786	0.0810
6	180	0.0963	0.0940

Note: The test sample were withdrawn at the time interval as mentioned and scanned in UV using phosphate buffer as blank

SAPONIFICATION VALUE

Sample was treated as mentioned in the monograph and evaluated for the saponification value. The value was found to be **349.92mg** of KOH/g. Normal range of value is **0 to 370 mg** KOH/g.

Marketed sample: saponification value result of formulated cream is **22.2**

ACID VALUE

The acid value determination of the sample was performed as per the monograph and it was found to be **1.6**. Normal range of value is **0.50 mg** KOH/g

Marketed sample: Acid value result of formulated cream is **4.5**

VISCOSITY TEST

The viscosity of the cream is measured to determine its thickness and flow properties. This test is typically performed using a viscometer or rheometer. A cream with appropriate viscosity should spread easily on the skin without being too runny or too thick. The ideal viscosity ensures ease of application and good skin coverage while maintaining the stability of the formulation. Measurements are taken at room temperature, and the results are compared to standard values for similar products.

Name of the Sample: Spirulina Cream

Date of Analysis: 04/07/2004

Type of viscometer: DV2T Brookfield viscometer

Type of Spindle: Spindle No. LV 63

Speed: 0.5 RPM

Temperature: 33° C

Table No. 5.

Name of sample	Trial	Viscosity
Spirulina cream	1	1,07,800cp
	2	1,14,200cp
	3	1,26,700cp
	Average	**1,16,233cp**

The Viscosity of the Spirulina cream is **1,16,233cp,** The normal range of viscosity is **200 to 6000** cP at 25°C.

Marketed sample: The viscosity of cream was the range of **499990 to 30000 cp** which indicates that the cream is easily spreadable by small amount of shear. The formulated cream shows the viscosity within range **48880cp.**

ANTI-MICROBIAL TEST

The anti-microbial test evaluates the cream's ability to inhibit the growth of microorganisms. This is essential for ensuring the product's safety and effectiveness, particularly if it contains natural ingredients that may be prone to microbial contamination. The test typically involves the following steps:

1. **Sample Preparation:** A specified amount of the cream is prepared for testing.
2. **Microbial Inoculation:** The cream is inoculated with a known quantity of different microorganisms, including bacteria and fungi, commonly found in cosmetic products.
3. **Incubation:** The inoculated cream is incubated at specific temperatures for a set period, usually 24 to 48 hours, to allow microbial growth.
4. **Assessment:** The number of viable microorganisms is assessed using methods such as plate counting, where samples are spread on agar plates and the resulting colonies are counted.
5. **Comparison:** The results are compared to control samples to determine the extent of microbial inhibition. A significant reduction in the number of viable microorganisms indicates effective anti-microbial properties.

The anti-microbial test ensures that the cream is safe for use and has a sufficient shelf life by preventing the growth of harmful microorganisms.

Name of the Sample: Spirulina Cream

Date of Analysis: 10/07/2024-11/07/2024

OBJECTIVE

To evaluate the anti-microbial activity of the herbal moisturizing cream by determining its ability to inhibit the growth of specific bacteria using the cup plate method.

METHODOLOGY

- **Method of Study:** Cup Plate Method
- **Medium:** Mueller-Hinton Agar
- **Standard:** Ofloxacin
- **Incubation Period:** 24 hours
- **Temperature:** 35°C

Microorganisms Tested

1. **Bacillus subtilis** (NCIM 2063)
2. **Staphylococcus aureus** (NCIM 2901)
3. **Escherichia coli** (NCIM 2345)
4. **Pseudomonas aeruginosa** (NCIM 5029)

Sample Preparation and Testing

- Bacterial cultures were spread uniformly on Mueller-Hinton Agar plates.
- Wells were cut into the agar, and 0.1 ml of the cream sample was added to each well.
- Ofloxacin was used as the standard reference for comparison.
- Plates were incubated at 35°C for 24 hours.
- Zones of inhibition were measured in millimetres (mm) after the incubation period.

Table No. 6.

S. No	Name of the Microorganism	NCIM	Zone of Inhibition (mm)							
			Standard				Test			
			1	2	3	Average	1	2	3	Average
1.	*Bacillus subtilis*	2063	20	23	23	22	8	7	7	7.3
2.	*Staphylococcus aureus*	2901	17	20	13	16.6	5	6	6	5.6
3.	*Escherichia coli*	2345	24	26	24	24.6	5	5	5	5.0
4.	*Pseudomonas aeruginosa*	5029	18	17	17	17.3	6	5	5	5.3

Fig.no.2.

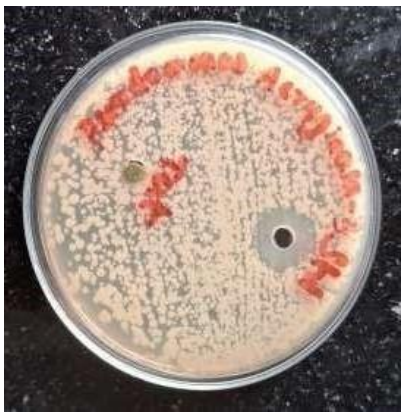

Fig.no.3.

Fig.no.4.

Fig.no.5.

Fig.no.6.

Fig.no.7.

Fig.no.8.

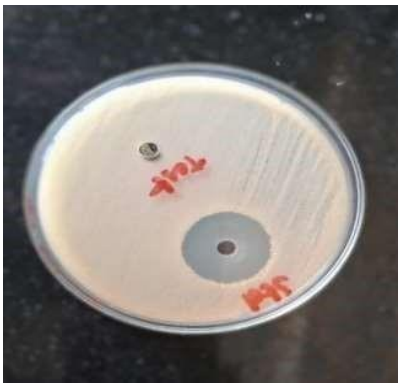

Fig.no.9.

FINAL RESULTS AND DISCUSSION

Results

- **Bacillus subtilis:** The herbal moisturizing cream exhibited a zone of inhibition that is lower than that of the standard Ofloxacin, indicating reduced anti-microbial activity against Bacillus subtilis.
- **Staphylococcus aureus:** The cream showed a smaller zone of inhibition compared to the standard, reflecting decreased effectiveness against Staphylococcus aureus.
- **Escherichia coli:** The inhibition zone for the cream was significantly smaller than that of the standard, suggesting diminished effectiveness against Escherichia coli.
- **Pseudomonas aeruginosa:** The cream demonstrated moderate anti-microbial activity, with a smaller inhibition zone relative to the standard.

DISCUSSION

The herbal moisturizing cream shows some anti-microbial activity against the tested microorganisms but is less effective compared to the standard Ofloxacin. The cream's lower inhibition zones indicate that its anti-microbial properties are not as robust as those of the standard. This suggests that the current formulation provides only moderate microbial protection. To improve efficacy, further enhancements to the formulation or the inclusion of more potent antimicrobial agents may be necessary.

CONCLUSION

The evaluation of the herbal moisturizing cream reveals several important insights. The cream, formulated with ingredients like spirulina, vitamin E oil, aloe vera gel, and neem oil, was prepared with careful attention to the combination of oil and water phases, following specific temperature guidelines for effective emulsification. It generally maintained a smooth, uniform texture, free from lumps or separation, and was well-tolerated in skin irritation tests, indicating good formulation quality. Washability tests showed that the cream can be effectively removed without significant residue, contributing to user comfort. Viscosity measurements confirmed that the cream has an appropriate consistency for application, enhancing the overall user experience.

However, the anti-microbial activity of the cream was less effective compared to the standard Ofloxacin. The cream displayed lower inhibition zones against microorganisms such as Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. This suggests that while the cream offers some degree of microbial protection, its efficacy is limited. Further formulation adjustments are recommended to improve its anti-microbial effectiveness. Overall, while the cream shows a generally favorable profile in physical attributes and skin compatibility, enhancing its anti-microbial properties is crucial for achieving optimal quality and efficacy.

REFERENCES

1. Macuh M, Knap B. Effects of nitrate supplementation on exercise performance in Humans: a narrative review. Nutrients, 2021; 13: 3183. Doi: 10.3390/nu13093183
2. Kluge B, Krupp L, Enoka R. Fatigue and fatigability in neurologic illnesses: proposal for a unified taxonomy. Neurology, 2013; 80: 409–16. Doi: 10.1212/WNL.0b013e31827f07
3. S. Hosseini, S. Shahbazizadeh, K. Khosravi-Darani, M. Mozafari Spirulina paltensis: food and function Curr Nutr Food Sci, 2013; 9(3): 189-193, 10.2174/1573401311309030003
4. Ismail, M., Hossain, M. F., Tanu, A. R. & Shekhar, H. U. Effect of Spirulina Intervention on Oxidative Stress, Antioxidant Status, and Lipid Profile in Chronic Obstructive Pulmonary Disease Patients. Biomed. Res. Int., 2015; 2015: 1–7.
5. Komárek J, Hauer T. CyanoDB.cz—On-line database of cyanobacterial genera. Worldwide electronic publication, Univ. of South Bohemia and Inst of Botany AS CR 2009.
6. Habib MAB, Parvin M, Huntington TC, Hasan MR. A review on culture, production, and use of Spirulina as food for humans and feeds for domestic animals and fish. FAO Fisheries and Aquaculture Circular No.034, 2008.
7. Blé-Castillo JL, Rodríguez-Hernández A, Miranda-Zamora R, Juárez-Oropeza MA, DíazZagoya JC. Arthrospira maxima prevents the acute fatty liver induced by the administration of simvastatin, ethanol and a hypercholesterolemic diet to mice. Life Sci, 2002; 70: 2665–2673.
8. Weltman MD, Farrell GC, Hall P, Ingelman-Sundberg M, Liddle C. Hepatic cytochrome P450 2E1 is increased in patients with nonalcoholic steatohepatitis. Hepatology, 1998; 27: 128–133.
9. Dasgupta T, Banejee S, Yadav PK, Rao AR. Chemomodulation of carcinogen metabolising enzymes, antioxidant profiles and skin and forestomach papillomagenesis by Spirulina platensis. Mol Cell Biochem, 2001; 226: 27–38.
10. Opperman, L.; De Kock, M.; Klaasen, J.; Rahiman, F. Tyrosinase and Melanogenesis Inhibition by Indigenous African Plants: A Review. Cosmetics, 2020; 7: 60.

11. Knott A, Reuschlein K, Mielke H, Wensorra U, Mummert C, Koop U, Kausch M, Kolbe L, Peters N, Stab F. et al. Natural Arctium lappa fruit extract improves the clinical signs of aging skin. J Cosmetic Dermatol, 2008; 7(4): 281–289. Doi: 10.1111/j.14732165.2008.00407.
12. Paulsen E. Contact sensitization from Compositae-containing herbal remedies and cosmetics. Contact Dermatitis., 2002; 47(4): 189–198. Doi: 10.1034/j.16000536.2002.47040.
13. Purnamawati S, Indrastuti N, Danarti R, Saefudin T., The role of moisturizers in addressing various kinds of dermatitis: a review clin Med Res, 2017; 15(3): 75.87.
14. Niki E, Abe K. Vitamin E: Structure, properties and functions, 2019 ebookcollection, Food Chemistry, Function and Analysis, Kouichi Abe, 2019.
15. Al-Busaid MM, Akhtar MS, Alam T, Shehata WA., Development and evaluation of herbal cream containing Curcumin from Curcumalonga. Pharm Pharmacol Int J., 2020; 8(5): 285-289.
16. Muthukumarasamy R, Ilyana A, Fithriyaani NA, Najihah NA, Asyiqin N, Sekar M., Formulation and evaluation of natural antioxidant cream comprising methanolic peel extract of Dimocarpus longan. Int.J.Pharm. Clin.Res, 2016; 8(9): 13051309.
17. A Priyanka Sharma, Amit C Kharkwal, Harsha Kharkwal, MZ Abdin, Ajit Varma. A review on the pharmacological properties of Aloe Vera. Int J Pharm Sci Rev Res, 2014; 29: 31-7.
18. Prall JK, Theiler RF, Bowser PA, Walsh M. The effectiveness of cosmetic products in alleviating a range of skin dryness conditions as determined by clinical and instrumental techniques. Int J Cosmet Sci., 1986; 8: 159–74.
19. Bhakti Mali, Sumedh N Moharil, Vaibhav Mhasal, Mahesh B Narkhede. Drug-excipient interaction study of Tramadol HCL with polymers. World J Pharm Res, 2017; 6: 848-61.
20. Khan, Z., Bhadouria, P., & Bisen, P. S. “Nutritional and therapeutic potential of Spirulina”. Current Pharmaceutical Biotechnology*, 2005; 6(5): 373-379. Doi:10.2174/138920105774370607.
21. Becker, E. W., “Micro-algae as a source of protein”. *Biotechnology Advances*, 2007; 25(2): 207-210. Doi:10.1016/j.biotechadv.2006.11.002.
22. Marles, R. J., & Barrett, M. L., “Pharmacognosy of Spirulina (Arthrospira): An overview of its biological activities and potential health benefits”. *Phytochemistry Reviews*, 2013; 12(3): 555-567. Doi:10.1007/s11101-013-9303-3.
23. Kuriakose, G. C., & Kurup, M. G., “Enhancement of skin health using Spirulina: Study on the effect of topical application”. *Journal of Applied Phycology*, 2010; 22(1): 85-89. Doi:10.1007/s10811-009-9441-7.
24. Chamorro, G., Salazar, M., & Favila, L., “Pharmacology and toxicology of Spirulina alga”. *Revista de Investigacion Clinica*, 2002; 54(5): 385-393.
25. Wu, L. C., Ho, J. A. A., Shieh, M. C., & Lu, I. W., “Antioxidant and antiinflammatory activities of cyanobacteria-derived extract from Spirulina platensis”. Journal of Agricultural and Food Chemistry*, 2005; 53(10): 3455-3462. Doi:10.1021/jf047956j.