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Formulation and characterization of a cooling sunscreen incorporated with chitosan-encapsulated spirulina

Ganesh Sanker S, Joel Samuel Koshy *, Sreelekshmi S M and Sunwin S

Department of Pharmaceutics, Mar Dioscorus College of Pharmacy, Alathara, Thiruvananthapuram.

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Abstract

Exposure to ultraviolet radiation can disrupt the normal balance of skin, leading to increased oxidative damage and accelerated photoaging (aging associated with light) and increased risk of developing skin cancer. To address these issues, it has become increasingly important to develop more effective, stable, and protective photoprotective products. The use of traditional sunscreens provides an adequate barrier against UV rays but they are known to cause skin irritation, be unstable when exposed to heat and moisture, and reduced acceptability by consumers. Spirulina contains phycocyanin and various natural antioxidants, presents strong photoprotective and anti-inflammatory potential; however, its poor stability and low penetration hinder direct incorporation into formulations. This paper deals with the encapsulation of Spirulina extract by the ionic gelation technique, using chitosan nanoparticles to provide higher stability, better retention of antioxidants, and sustained release. These nanoparticles are combined into an optimized oil-in-water cooling sunscreen cream containing both mineral and chemical UV filters, such as ZnO, TiO_2, Avobenzone, and Octocrylene, with soothing agents like Panthenol and Menthol.

The developed sunscreens were tested for their physicochemical properties, SPF values, antioxidant activity, stability, rheology, spreadability, and microbiological safety. This study is expected to highlight the fact that chitosan-encapsulated Spirulina can act as an excellent natural SPF booster and antioxidant, improving photoprotection along with enhanced skin comfort and reduced irritation. This proposed formulation should be capable of offering a multifunctional, stable, cosmetically acceptable cooling sunscreen with broad-spectrum UV protection in addition to therapeutic benefits.

Keywords: Chitosan nanoparticles; Spirulina; Photoprotection; Nanoencapsulation; Ionic gelation; UV filters

1. Introduction

One of the major causes of the occurrence of different types of damage to the skin, such as oxidative stress, aging, and carcinogenesis, is the damage caused by ultraviolet rays. The skin is an important organ that provides protection against different types of environmental hazards, but the damage caused by ultraviolet rays may affect the structural and functional integrity of the skin [1,2]. The damage caused by UVB rays is related to the damage caused to the epidermis, and this type of damage is related to DNA damage, while the damage caused by UVA rays penetrates the dermis and causes the production of ROS, leading to photoaging and damage to collagen and elastin fibers [3, 4].

Sunscreens are known to play an important role in protecting the skin against damage caused by UV-induced effects. These are used as a first line of defence against damage, either by absorbing or reflecting the damaging rays. Broad-spectrum sunscreens are known to contain both inorganic and organic filters, with zinc oxide and titanium dioxide being used along with avobenzone and octocrylene to give an effective level of protection against UVA and UVB rays [5,6].

* Corresponding author: Joel Samuel Koshy

However, some limitations have been associated with the use of conventional sunscreen formulations. These limitations include photoinstability, skin irritation, and inadequate protection against oxidative stress [7,8,9].

In recent times, interest has been shown in using natural bioactive compounds to improve the efficacy of sunscreen formulations. Spirulina platensis is known to be a microalga that is rich in antioxidants. These have shown significant free radical scavenging and anti-inflammatory properties. Spirulina is known to reduce oxidative damage and provide better cellular protection against UV-induced effects [10,11,12]. However, the use of Spirulina is limited due to its stability problems and ease of degradation. Also, the skin penetration of Spirulina is limited. Therefore, nanotechnology-based delivery systems have been found to be an effective method to overcome these problems. Chitosan-based nanoparticles have been found to have better biocompatibility, stability, skin adhesiveness, and release properties. Therefore, the encapsulation of Spirulina using chitosan-based nanoparticles is expected to provide better stability to the bioactive compounds and improve skin adhesiveness [13,14]. Thus, the objective of the current study is to develop and evaluate a novel sunscreen formulation using chitosan-based nanoparticles containing Spirulina extracts along with hybrid sunscreen filters to provide improved photoprotection, antioxidant activity and provide a stable formulation.

2. Materials and method

2.1. Materials required

Table 1 List of materials

Sl. No.	Materials
1	Zinc oxide
2	Titanium dioxide
3	Avobenzone
4	Octocrylene
5	Chitosan flakes
6	Liquid paraffin
7	Emulsifying wax
8	Glycerine
9	Panthenol
10	Menthol
11	Phenoxyethanol
12	Spirulina powder

The active ingredients used were Chitosan, Spirulina powder, Zinc oxide, Titanium dioxide, Avobenzone, and Octocrylene. The excipients, such as emulsifying agents, preservatives, and humectants, were of analytical grade and used as such. The chemicals and reagents used in this study are of pharmaceutical or analytical grade.

2.2. Preparation of spirulina extract [15]

The extraction process of bioactive proteins, including C-phycocyanin, from Spirulina can be efficiently conducted by applying multiple cycles of freeze/thaw treatment followed by ammonium sulphate precipitation. The freeze-thaw method is a mild cell disruption technique that helps release intracellular pigments and proteins with minimal degradation.

In this technique, dried Spirulina biomass is mixed with a phosphate buffer (pH 6.8-7.2) and then frozen at -20°C. After that, it is thawed at room temperature. The ice crystals formed during the freezing process break the cell walls of the Spirulina biomass, thus aiding the release of the cell contents. This technique is followed multiple times, resulting in an increase in the efficiency of the extraction process and the yield of proteins.

This procedure is preferred because it is simple and economical. The freeze-thaw treatment followed by ammonium sulphate precipitation was found to be effective in recovering C-phycocyanin with high purity, making it fit for pharmaceutical or cosmetic industry requirements.

2.3. Formulation of sunscreen

- **Preparation of Chitosan-Encapsulated Spirulina Nanoparticles:** Chitosan nanoparticles encapsulating Spirulina platensis extract were prepared using the ionic gelation method with sodium tripolyphosphate as the crosslinker [16,17].
- **Preparation of chitosan solution**: Chitosan at a concentration of 0.2-0.5% w/v was accurately weighed, and a solution was prepared by dissolving chitosan in 1% v/v acetic acid with constant magnetic stirring for 4-6 hours, during which time a clear, homogeneous solution formed. The pH of the solution was adjusted to 4.5-5.0 with 0.1N NaOH to facilitate solubility [16].
- **Preparation of sodium tripolyphosphate solution**: Sodium tripolyphosphate at a concentration of 0.5-1% w/v was dissolved in 30 ml of distilled water and stirred to form a clear solution [17].
- **Addition of Spirulina extract**: Spirulina extract was slowly added to the chitosan solution with constant stirring by using a mechanical homogenizer at a rate of 800-1000 rpm to uniformly distribute the extract within the chitosan matrix [18].
- **Formation of nanoparticles through ionic gelation**: The solution of TPP is slowly added, drop by drop, to the chitosan-Spirulina mixture while continuously stirring. The nanoparticles will be formed spontaneously due to the electrostatic attraction that occurs between the positively charged amino groups of chitosan and the negatively charged groups of sodium tripolyphosphate [16,17].
- **Stirring and stabilization**: The formed nanoparticles are kept under constant motion for 1-2 hours at room temperature to allow for complete ionic cross-linking [16].
- **pH adjustment**: The pH of the formed nanoparticles is adjusted to a range of 5.5-6.0 by adding a solution of NaOH, which makes the system more stable for topical application [18].
- **Storage**: The chitosan-encapsulated spirulina nanoparticles will be stored in a container at 4°C before being used for the formulation of sunscreen cream.
- **Preparation of oil phase**: Zinc oxide, titanium dioxide (physical filters), Avobenzone and Octocrylene (chemical filters), Liquid paraffin (emollient/occlusive agent), and emulsifying wax were accurately weighed and mixed in a clean beaker. The mixture was then heated to 70°C with constant stirring until the emulsifying wax was completely melted, resulting in the uniform dispersion of the sunscreen filters [19,21].
- **Preparation of aqueous phase**: Glycerin (humectant), Panthenol and distilled water were accurately measured and mixed well. The mixture was then subjected to heating at 70 °C, like the oil phase, because the temperature of the two phases must be the same for the emulsion to be stable [20].
- **Emulsification:** The water phase that has been warmed was trickled into the oil phase while the mixer was still spinning at 1000-1500 rpm. This ensured that there was steady stirring to create a stable emulsion of oil in water. Both phases are also at the same temperature to prevent the mixture from solidifying early on and create an emulsion of uniform particle size [19].
- **Addition of heat-sensitive ingredients:** The emulsion is left to cool while still stirring slowly. When the emulsion drops below 40 degrees Celsius, add the chitosan-encapsulated Spirulina nanoparticles to protect the mixture from damage due to heat. We then add the menthol for the cooling effect and the Phenoxyethanol for preservation purposes while continuing to stir the mixture to ensure that all the ingredients are well mixed [22].
- **Final homogenization and storage:** The final cream product is then homogenized one more time for the best results, packed into containers, and stored at room temperature for later use.

Table 2 Formulation of sunscreen (50 g batch)

Ingredient	Function	F1 (SPF 30)	F2 (SPF 40)	F3 (SPF 50)
ZnO	Physical UV filter	7 g	8 g	9 g
TiO_2	Physical UVA/UVB filter	1.5 g	2 g	3 g
Avobenzone	Chemical UVA filter	1.5 g	2 g	2 g
Octocrylene	Chemical UVB filter	2 g	2.5 g	3 g

Chitosan-encapsulated Spirulina (10% active)	Antioxidant / SPF booster	5 ml	7.5 ml	10 ml
Emulsifying wax	O/W emulsifier	3.5 g	4 g	4.5 g
Liquid paraffin	Oil phase	3.5 g	4 g	4.5 g
Glycerine	Humectant	2g	2g	2g
Panthenol	Cytoprotective / anti-irritant	0.5 g	0.5 g	0.5 g
Menthol	Cooling	0.12 g	0.15 g	0.2 g
Phenoxyethanol	Preservative	0.25 g	0.25 g	0.25 g
Distilled water	Vehicle	q.s. to 50 g	q.s. to 50 g	q.s. to 50 g

3. Evaluation methods

3.1. Preliminary tests of herbal ingredient (Spirulina) [23,24,25,26,27]

3.1.1. Tests for carbohydrates

- **Molisch's test:** A quantity of 2 ml of the test solution was taken in a clean test tube, and 2-3 drops of Molisch's reagent (α-naphthol in alcohol) were added and mixed gently. 1 ml of concentrated sulfuric acid was added down the sides of the test tube, which formed a separate layer. The formation of a violet or purple ring at the interface confirmed the test for carbohydrates.
- **Fehling's test:** Equal volumes of 1 ml Fehling's solution A and 1 ml Fehling's solution B which were combined. The sample solution was introduced to the mixture which then underwent heating in a boiling water bath for 5 to 10 minutes. The appearance of a brick-red precipitate proved the existence of reducing sugars in the sample.
- **Benedict's test:** About 2 ml of Benedict's reagent was taken in a test tube, and 1 mL of the sample solution was added. The mixture was heated in a boiling water bath for about 5 minutes and then allowed to cool. Formation of brick red precipitate indicates presence of reducing sugars.

3.1.2. Tests for proteins

- **Biuret test:** A test tube was filled with about 2 ml of the test solution. Then, 1 ml of a 10% sodium hydroxide solution was added to make the solution alkaline. Finally, 2-3 drops of a 0.1% copper sulphate solution were added.
- **Ninhydrin test:** 2 ml of the sample extract was taken in a test tube and a few drops of ninhydrin reagent were added. The mixture was then heated in a boiling water bath for 2-3 minutes. Formation of blue or purple colour indicated presence of protein.
- **Millon's test:** 2 ml of sample extract was taken in a test tube and few drops of Millon's reagent was added and heated in a water bath. Formation of red/ pink colour indicated the presence of proteins.

3.1.3. Tests for alkaloids

- **Dragendorff's test:** Add few drops of Dragendorff's reagent into test tube containing acidic extract of the sample. Formation of orange-brown precipitate indicates presence of alkaloids
- **Mayer's test:** 2 ml of the sample extract was taken in a test tube. And treated with few drops of Mayer's reagent. Formation of reddish-brown precipitate indicates the presence of alkaloids.
- **Wagner's test:** Few drops of Wagner's reagent were added to acidified sample extract. Formation of reddish-brown precipitate indicates the presence of alkaloids.

3.1.4. Tests for phenols

- **Ferric chloride test:** To 2 ml of sample extract taken in a test tube, few drops of ferric chloride solution were added. The formation of green/blue/purple colour indicates the presence of phenols.
- **Lead acetate test:** To 2 ml of sample extract taken in a test tube, few drops of lead acetate solution were added. The formation of white precipitate indicates the presence of phenols.

- **Vanillin HCl test:** 2 ml of the sample extract was treated with few drops of vanillin in ethanol and hydrochloric acid. Formation of red/pink colour indicates the presence of phenols.

3.1.5. Tests for flavonoids

- **Alkaline Reagent (NaOH) Test:** To 2 ml of sample extract in a test tube, few drops of 10% NaOH were added. The formation of a yellow colour was observed and disappearance of yellow colour on addition of dilute hydrochloric acid indicates presence of flavonoids.
- **Shinoda Test:** To 2 ml of sample extract add few magnesium turnings and hydrochloric acid. Development of pink or red colour indicates the presence of flavonoids

3.1.6. Tests for glycosides

- **Borntrager's Test:** 2ml of the sample extract was hydrolysed with dilute hydrochloric acid and filtered. Chloroform was added to the filtrate and separated. To this an equal amount of ammonia was added and shaken. Formation of pink or red colour indicates the presence of anthraquinone glycosides.
- **Keller–Killiani Test:** 2 ml of the sample extract was treated with 1ml glacial acetic acid, 5% ferric chloride and sulfuric acid. The formation of a brown ring at the interface indicated the presence of cardiac glycosides.
- **Baljet Test:** A small amount of sample extract was treated with few drops of sodium picrate (Baljet reagent). Formation of orange/red colour indicates the presence of cardiac glycosides.
- **Saponin test:** About 2 ml of the test solution was diluted with distilled water and shaken vigorously for about 15 minutes. The formation of a stable and persistent froth indicated the presence of saponins.

3.2. Physicochemical tests of herbal ingredient (Spirulina) [28,29,30,31,32]

- **Total ash value:** This test is used for determination of total amount of inorganic residues present in the sample. Weighed about 3 g of the sample and was incinerated in a silica crucible at a temperature of not exceeding 600 °C until a constant weight was obtained. The residue was cooled and weighed. The total ash value was calculated as a percentage of air-dried sample, representing the total mineral content including physiological and non-physiological ash.
- **Acid-Insoluble ash value:** The acid-insoluble ash test was conducted to find out the amount of silica and earthy matter present. The total ash content obtained was boiled in dilute hydrochloric acid, filtered, and the acid-insoluble matter was collected on ashless filter paper. It was washed, dried, and then incinerated. It was finally weighed, and the acid-insoluble ash value was calculated as a percentage of the air-dried sample.
- **Water-Soluble ash value:** Water-soluble ash content was determined to find out the amount of water-soluble inorganic salts present. The total ash content was boiled with distilled water, and the mixture was filtered. The amount of insoluble matter collected was then incinerated. The weight of the insoluble matter collected was subtracted from the total ash content.
- **Moisture Content (Loss on Drying):** The moisture content was determined using the loss of drying method. A known quantity of Spirulina powder was accurately weighed and dried in an oven at 105°C until a constant weight was achieved. The loss in weight was calculated and expressed as a percentage, showing the moisture content present in the sample.
- **Water-Soluble Extractive Value:** Water-soluble extractive value was determined in order to estimate the amount of water-soluble compounds present in the sample. About 5 g of air-dried spirulina powder was macerated with 100 ml of distilled water with frequent shaking at room temperature for 24 hours. The mixture was filtered, and 25 ml of the filtrate was evaporated to dryness in a pre-weighed dish and dried at 105 °C. The dry weight was taken, and the extractive value was expressed as a percentage of the air-dried sample.
- **Alcohol-Soluble Extractive Value:** Alcohol-soluble extractive value was determined to estimate the amount of alcohol-soluble compounds present in the sample. About 5 g of the sample was macerated with 100 ml of alcohol (ethanol) with frequent shaking at room temperature for 24 hours. The solution was filtered, and 25 ml of the filtrate was evaporated to dryness in a pre-weighed dish and dried at 105 °C. The dry weight was taken, and the extractive value was expressed as a percentage of the air-dried sample.

3.3. Physicochemical and Performance Evaluation of Sunscreen Cream [33,34,35]

- **Organoleptic Evaluation:** The formulated sunscreen cream was assessed for its organoleptic properties, including colour, odour, appearance, and texture. The formulated sunscreen cream was visually assessed for uniformity, consistency, and any phase separation and grittiness. The parameters are useful in assessing the quality of the formulated sunscreen cream.

- **Determination of pH:** The pH value of the sunscreen cream was determined to assess its suitability for the skin. A sample of the sunscreen cream, approximately 1g, was mixed with 10 ml of distilled water. After 2 hours, the pH value of the mixture was measured using a calibrated digital pH meter. The test was conducted at room temperature. The pH value of the mixture was recorded.
- **Spreadability test:** The spreadability of the formulated sunscreen cream was determined by the glass slide method. The glass slide method involves applying a known amount of formulated sunscreen cream between two glass slides and compressing it to a uniform thickness by applying a known weight for a fixed period. After applying a known weight for a fixed period, the diameter of the spread sunscreen cream is determined. The spreadability is determined by applying the formula:

$$S = (d^2 \times \pi) / 4$$

Where S is spreadability in mm^2, and d is the diameter of the spread sunscreen cream in cm or mm. The spreadability is a measure of the spread of the sunscreen cream. The higher spreadability is an indication of better spreadability and application of the formulated sunscreen cream.

- **Determination of viscosity:** The viscosity of the sunscreen cream formulation was determined with the help of a Brookfield viscometer, which was set to room temperature with a specific spindle rotating at a fixed rate. The viscosity readings of the cream formulation were noted in centipoise (cP), which reflects the viscosity of the cream.
- **Homogeneity Test:** For the homogeneity of the formulation, the mixture was inspected visually and rubbed between the fingers. The inspection was done to ascertain the homogeneity of the mixture. A homogeneous mixture shows that the mixture was properly mixed and is stable.
- **Determination of emulsion type:** The dilution test was conducted to identify the nature of the emulsion used in the formulation. The test was conducted by adding a small amount of the sunscreen cream to distilled water, which was later observed to check its miscibility. If the formulation mixed uniformly with water, it was identified as an oil-in-water emulsion. To further identify the nature of the emulsion, the sunscreen cream formulation was mixed with an oily phase, which was represented by liquid paraffin. If the formulation did not mix uniformly with the oily phase, the emulsion was identified as an oil-in-water emulsion. Emulsions that are easily miscible with an oily phase but not with water are identified as water-in-oil emulsions.

3.4. *In vitro* Antioxidant Study [36,37,38]

Antioxidants are found to play a significant role in defending biological systems against oxidative stress induced by free radicals. Incorporation of natural bioactive agents such as Spirulina into sunscreen formulations can enhance their protective efficacy by providing additional antioxidant benefits. Hence, it is important to note that the antioxidant activity of the sample has been evaluated by performing a DPPH free radical scavenging assay and a Ferric Reducing Antioxidant Power assay, as they are reliable methods for evaluating free radical scavenging activity.

3.4.1. DPPH Radical Scavenging Assay

- **Preparation of Reagents:** A solution of 0.1 mM DPPH was prepared by accurately weighing DPPH and dissolving it in methanol. The solution was stored in an amber-coloured bottle to prevent exposure to light. Methanol was used as a blank.
- **Preparation of Sample Solution:** An accurately weighed quantity of the formulated sunscreen cream was taken and extracted using methanol. From the stock solution, a series of solutions with different concentrations (20, 40, 60, 80, and 100 µg/ml) were obtained by dilution.
- **Procedure:** To 1 ml of each concentration of the sample extract, 2 ml of DPPH solution was added, which was incubated in the dark at room temperature for 30 minutes. The absorbance was recorded at 517 nm using a UV-Vis spectrophotometer. The control sample was prepared by adding DPPH solution with methanol instead of the sample extract.

Calculation

The percentage inhibition of DPPH radicals was calculated using the formula:

$$\text{Percentage Inhibition} = [(A_0 - A_1) / A_0] \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

3.4.2. Ferric Reducing Antioxidant Power (FRAP) Assay

The antioxidant activity of the formulated sunscreen was determined by measuring its ability to donate electrons to reduce ferric ions to ferrous ions. The reaction is accompanied by the formation of a coloured compound, where the intensity of the colour is a function of the antioxidant activity of the sample.

- **Preparation of Buffer Solution:** A buffer solution of 0.2 M concentration and a pH of 6.6 was prepared by mixing a known amount of sodium dihydrogen phosphate monohydrate with distilled water. The solution was then adjusted to the desired pH with a sodium hydroxide solution before making it up with water.
- **Preparation of Sample Extract:** The sunscreen formula was extracted with methanol with constant stirring to ensure efficient recovery of active ingredients. The solution was then filtered, and the filtrate obtained was used as a stock solution. Further solutions were prepared to get different concentrations.

Procedure: An aliquot of 1 ml of the prepared sample solution was mixed with 2.5 ml of phosphate buffer (pH 6.6), and then 2.5 ml of potassium ferrocyanide solution (1% w/v) was added. The mixture was then placed in a water bath at 50°C for 20 minutes.

2 ml of 10% trichloroacetic acid was then added to the mixture, and 2.5 ml of the mixture was then mixed with an equal volume of distilled water and 0.5 ml of ferric chloride solution (0.1% w/v). The bluish-green coloration of the mixture showed the presence of reducing agents.

The absorbance of the solution was then measured at 700 nm by a UV-Vis Spectrophotometer. Ascorbic acid was used as a reference standard, and distilled water was used as a blank.

3.5. Characterization studies of sunscreen formulation [39,40,41]

3.5.1. FTIR Analysis (Fourier Transform Infrared Spectroscopy)

FTIR analysis was done to ascertain the functional groups present in the formulation and evaluate the compatibility of the active ingredients with the excipients in the sunscreen formulation. The results of the analysis provide information regarding the possible chemical interaction of the compounds with the aid of the characteristic peaks of the compounds compared with the peaks of the formulation. The absence of a change in the peaks or the disappearance of the characteristic peaks indicates the compatibility of the formulation.

3.5.2. XRD Analysis (X-ray Diffraction)

XRD analysis was done to determine the crystalline or amorphous nature of the compounds present in the formulation. The XRD peaks indicate the changes in the crystalline nature of the compounds due to the formulation process such as encapsulation or emulsification. The decrease in the intensity of the peaks or the appearance of broad peaks indicate the change in the nature of the compounds towards the amorphous form, which improves the solubility of the active compounds.

3.6. Determination of Sun Protection Factor (SPF) by UV Spectrophotometry [42,43]

Sun Protection Factor (SPF) is a key factor used to assess the efficiency of sunscreen creams in protecting human skin from exposure to ultraviolet radiation, especially UVB radiation. The SPF of the prepared sunscreen cream was determined by using the in vitro method of UV-Vis Spectrophotometry, based on the Mansur equation, which is a simple and accurate method for measuring SPF values of sunscreen products.

- **Preparation of sample solution:** 1g of sunscreen cream was dissolved in a suitable solvent, such as ethanol or methanol, was introduced to achieve an appropriate concentration, for instance, 0.2 mg/ml. Continuous stirring was then implemented to facilitate the extraction of the UV-absorbing constituents. The resulting solution was filtered to remove any insoluble material.
- **Procedure:** The prepared sample solution was scanned in the UV range of 290 to 320 nm with a scan interval of 5 nm, i.e., 290, 295, 300, 305, 310, 315, and 320 nm, with the help of a UV-VIS spectrophotometric instrument. The absorbance of the sample solution was noted.
- **Procedure:** The SPF of the sunscreen formulation was calculated with the help of Mansur's equation, which is as follows:

$$SPF = CF \times \Sigma [EE(\lambda) \times I(\lambda) \times Abs(\lambda)]$$

In the above equation, the following parameters are used:

- CF – correction factor, which is generally taken as 10
- EE(λ) – erythemal effect spectrum
- I(λ) – solar intensity spectrum
- Abs – absorbance of the sample solution
- Standard EE × I values are used to calculate SPF.

Inference: It has been inferred that higher SPF values indicate good protection of the formulation against UVB rays. The method offers a way to estimate the efficacy of a sunscreen formulation.

4. Results and discussions

4.1. Preparation of sunscreen

Three formulations of chitosan-encapsulated spirulina sunscreen of formulations F1, F2 and F3 were prepared.

Figure 1 Prepared sunscreen formulations (F1, F2, F3)

4.2. Preliminary tests of herbal ingredient (Spirulina)

Preliminary phytochemical screening of the herbal ingredient was carried out to detect the presence of various phytoconstituents using standard methods.

Table 3 Preliminary test of herbal ingredient (Spirulina)

Sl. No.	Phytoconstituent	Spirulina Extract
1	Carbohydrates	Positive
2	Reducing sugars	Positive
3	Proteins / Amino acids	Positive
4	Alkaloids	Negative
5	Phenolic compounds	Positive
6	Flavonoids	Positive
7	Glycosides	Negative
8	Saponins	Positive

4.3. Physicochemical tests of herbal ingredient (Spirulina)

Physicochemical tests for spirulina were performed, and the following results were obtained

Table 4 Physicochemical tests of herbal ingredient

Sl. No.	Parameter	Observed Value (% w/w)
1	Total Ash Value	7.28
2	Acid Insoluble Ash value	0.47
3	Water soluble ash value	4.23
4	Moisture content	5.69
5	Alcohol soluble extractive value	13.20
6	Water soluble extractive value	19.85

4.4. Physicochemical Evaluation of Sunscreen Formulation

All the prepared formulations (F1, F2, F3) of sunscreen were subjected to the following evaluation parameters.

4.4.1. Organoleptic Evaluation

All three formulations (F1, F2, and F3) of sunscreen were evaluated for physical evaluation, and the results are shown in Table No 5.3.

Table 5 Organoleptic evaluation

Sl. No.	Evaluation Parameters	Observations		
		F1	F2	F3
1	Visual appearance	White	White	White
2	Texture	Smooth	Smooth	Smooth
3	Odour	Characteristic	Characteristic	Characteristic

The results of the organoleptic analysis indicated that all the formulations (F1, F2, and F3) had similar appearance, texture, and characteristic odour. These properties are important in the acceptability of the product by the consumer, as they are part of the product's properties during its application. The smooth texture and characteristic fragrance are important properties of a cosmetic product, as they are meant for daily use, making sure the cream is not only effective but also pleasant to use.

4.4.2. pH determination

The pH of all three formulations (F1, F2, and F3) was recorded by a digital pH meter, and the results of pH were shown in Table 6.

Table 6 Determination of pH

Sl. No.	Formulation	pH
1	F1	6.22
2	F2	6.37
3	F3	6.59

Figure 2 Digital pH meter

The pH of the formulations was found to be in the range of **6.22–6.59**, which is the acceptable range of pH of the skin.

4.4.3. Spreadability

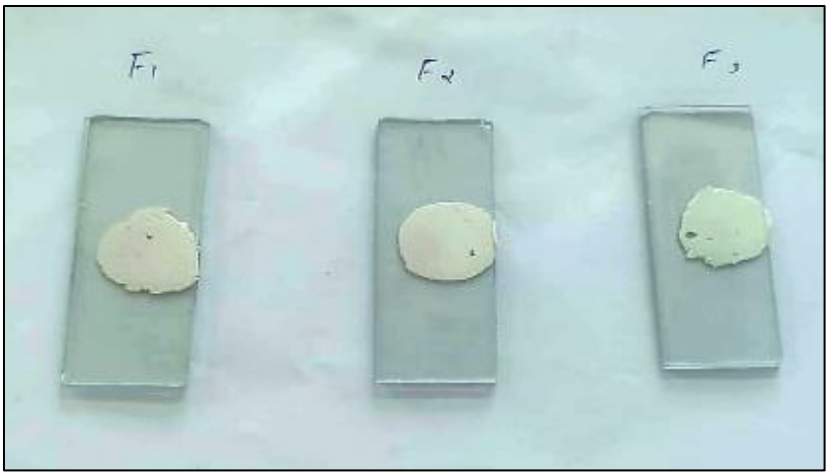

Figure 3 Spreadability of formulations

The spreadability of the formulated sunscreen creams was assessed by making use of the glass slide method, and the results are provided in Table 7. The spreadability of sunscreen creams F1, F2, and F3 were found to be 374.94 mm^2, 415.48 mm^2, and 346.36 mm^2, respectively. The spreadability of sunscreen cream F2 was found to be higher compared to F1 and F3.

All formulated sunscreen creams were found to possess satisfactory spreadability.

Table 7 Spreadability of formulations

Formulation	Diameter(cm)	Time(sec)	Spreadability($mm^{2)}$
F1	2.2 cm	300	374.94 mm^2
F2	2.3.cm	300	415.48 mm^2
F3	2.1.cm	300	346.36 mm^2

4.4.4. Viscosity

A Brookfield viscometer was used to measure the viscosity of each of the three sunscreen formulations. The result of viscosities is shown in Table 8.

Table 8 Measurement of viscosity

Sl. No.	Formulation	Viscosity(cps)
1	F1	10,223
2	F2	10,999
3	F3	11,143

Viscosities of all the formulations were noted and found in the range of 10,223 to 11,143 cps at 20 rpm, as shown in Table 8. All the formulations (F1, F2, and F3) had having acceptable range of viscosities.

4.4.5. Homogeneity

All the formulated sunscreen creams were evaluated for homogeneity, which was done through visual inspection as well as gentle application between the fingers. The formulations, F1, F2, and F3, were smooth, uniform in appearance, and free from lumps, grit, or separation, which implies that the formulations have good homogeneity with a good mixture of all the ingredients.

4.4.6. Determination of the type of emulsion

The dilution test was carried out to identify the type of emulsion present in the formulation. A small amount of the sunscreen cream was mixed with distilled water and observed. If the mixture mixed well and did not separate, it indicated the presence of an oil-in-water emulsion.

The test for the type of emulsion was also carried out by mixing the sunscreen cream with the oily phase, which was represented by the liquid paraffin. Since the sunscreen cream did not mix well with the oily phase, it confirmed the oil-in-water emulsion. Emulsions that mix well with the oily phase but not with the distilled water are known as water-in-oil emulsions.

4.5. *In vitro* Antioxidant Study

4.5.1. DPPH Radical Scavenging Assay

Table 9 DPPH scavenging activity of standard

Standard Concentration (µg/ml)	Absorbance	Percentage inhibition (%)
10	0.205	70.03
20	0.064	90.64
30	0.052	92.40
40	0.045	93.42
50	0.029	95.74

Table 10 DPPH scavenging activity of formulation

Sample	Absorbance	Percentage inhibition (%)
F1	0.341	77.27
F2	0.268	82.13
F3	0.244	83.73

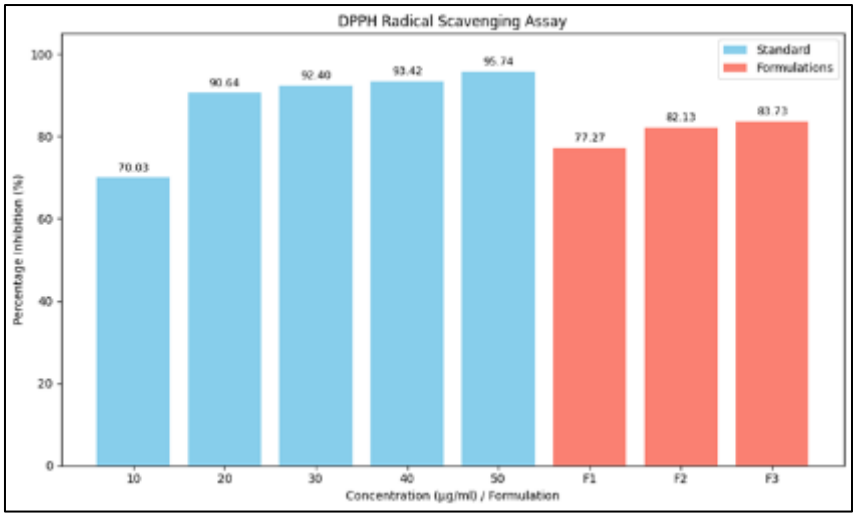

Figure 4 DPPH radical scavenging activity of standard and formulations

Antioxidant assay by DPPH radical scavenging activity was performed for standard and sample. All three formulations (F1, F2, and F3) show strong antioxidant activity, with % inhibition between 77–84%, indicating effective free radical scavenging. F3 shows the highest antioxidant activity.

4.5.2. Ferric Reducing Antioxidant Power (FRAP) Assay

Figure 5 Ferric reducing antioxidant power (FRAP) test

Table 11 FRAP assay of standard

Standard Concentration (ug/ml)	**Percentage Inhibition** (%)
20	65.17
40	74.18
60	78.66
80	81.31
100	96.42

Table 12 FRAP assay of formulation (50 µg/ml)

Formulation concentration (50µg/ml)	**Percentage Inhibition** (%)
F1	74.62
F2	78.43
F3	85.53

Table 13 FRAP assay of formulation (100µg/ml)

Formulation concentration (100µg/ml)	**Percentage Inhibition** (%)
F1	83.21
F2	88.60
F3	90.64

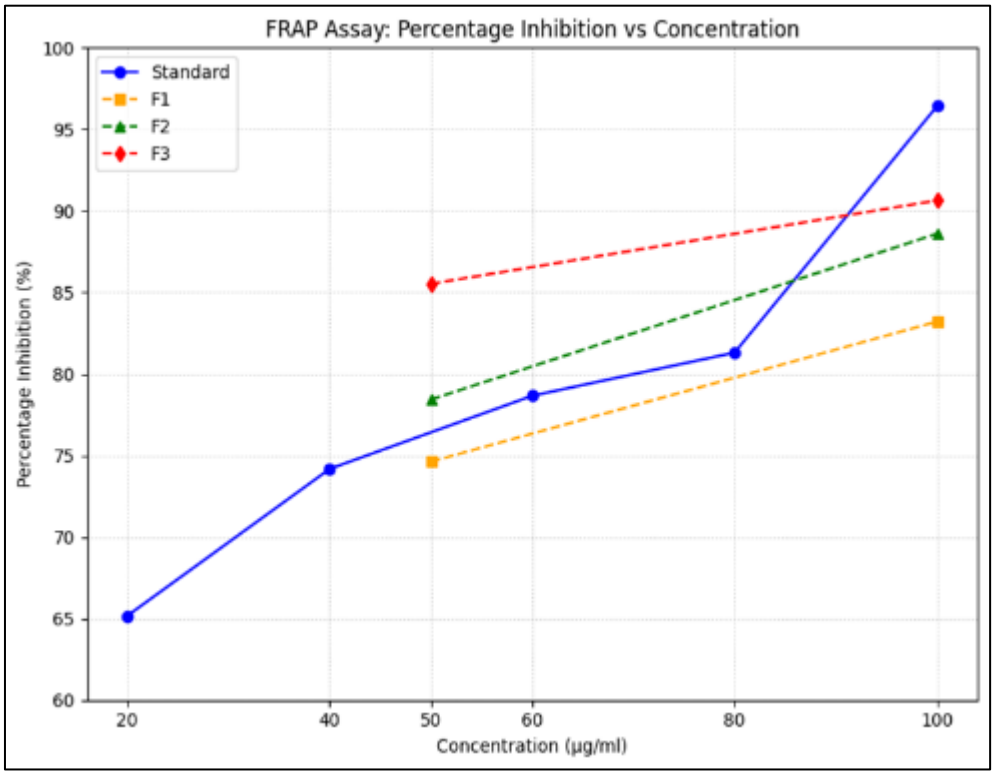

Figure 6 FRAP assay of standard and formulations

The results of the FRAP assay showed that all formulations (F1, F2, and F3) had antioxidant activity that depended on the concentration. F3 showed the highest percentage of inhibition at both 50 µg/ml and 100 µg/ml, indicating that it had the strongest reducing activity among the formulations tested.

4.6. Characterization studies of sunscreen formulation

4.6.1. FTIR Analysis (Fourier Transform Infrared Spectroscopy)

In the FTIR spectra, the formulations F1, F2, and F3 showed characteristic peaks for the major functional groups. The broad peak in the region 3200-3500 cm^{-1} indicates the O-H stretching. The peaks in the region 2850-2950 cm^{-1} correspond to the C-H stretching. The appearance of the peak in the region 1700-1750 cm^{-1} indicates the C=O

stretching. The appearance of the peaks in the region 1000-1300 cm^{-1} indicates the C-O stretching. The C=C stretching for the aromatic ring appears in the region 1450-1600 cm^{-1}.

There are no shift and disappearance of the peaks for the formulations F1, F2, and F3, indicating the absence of chemical interactions. This confirms the compatibility and stability of the formulation components.

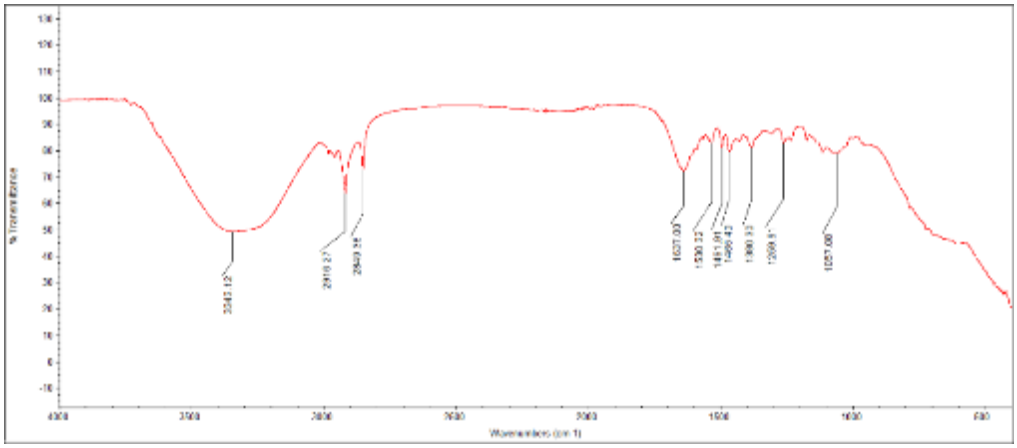

Figure 7 FTIR spectrum of sunscreen formulation F1

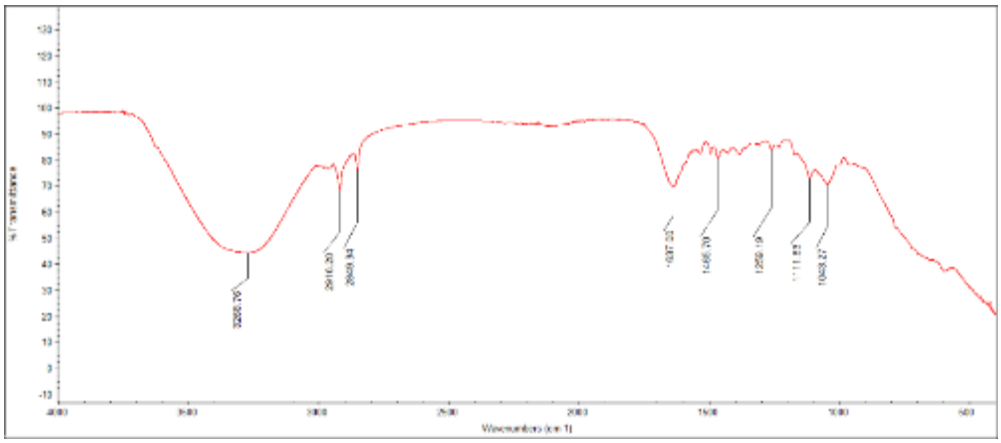

Figure 8 FTIR spectrum of sunscreen formulation F2

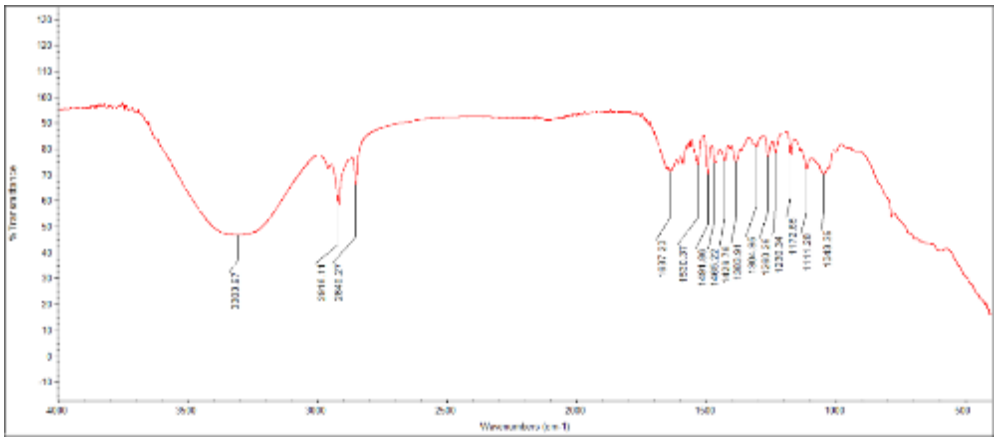

Figure 9 FTIR spectrum of sunscreen formulation F3

Table 14 FTIR peak assignment

Wavenumber (cm^{-1})	Functional Group	Interpretation
3200–3500	O–H stretching	Alcohols / moisture
2850–2950	C–H stretching	Alkanes (emollients, oils)
1700–1750	C=O stretching	Esters (UV filters, emulsifiers)
1450–1600	C=C stretching	Aromatic compounds
1000–1300	C–O stretching	Esters, ethers

Table 15 Comparative FTIR analysis of formulations

Parameter	F1	F2	F3
O–H stretching peak	Present	Present	Present
C–H stretching	Present	Present	Present
C=O stretching	Present	Present	Present
Aromatic C=C peaks	Present	Present	Present
Fingerprint region complexity	Moderate	Moderate	Slightly higher
Peak shift	Not observed	Not observed	Not observed
Chemical interaction	Not significant	Not significant	Not significant

4.6.2. XRD Analysis (X-ray Diffraction)

X-ray diffraction analysis of formulations F1, F2, and F3 was conducted to check the nature of the formulations, whether they are crystalline or amorphous in nature. The X-ray diffraction patterns of all the formulations showed characteristic peaks at different 2θ values.

This shows that all the formulations are partially crystalline in nature. The characteristic peaks obtained in all the formulations are of low intensity and are broadened compared to the characteristic peaks of pure crystalline materials. This shows that the ingredients are predominantly in the amorphous state. No significant changes in the characteristic peak positions are seen in formulations F1, F2, and F3. This shows that there are no new phases formed in the formulations, indicating that there are no interactions between the formulation ingredients.

This shows that the formulations are stable in nature, as all the ingredients are in the amorphous state or dispersed state.

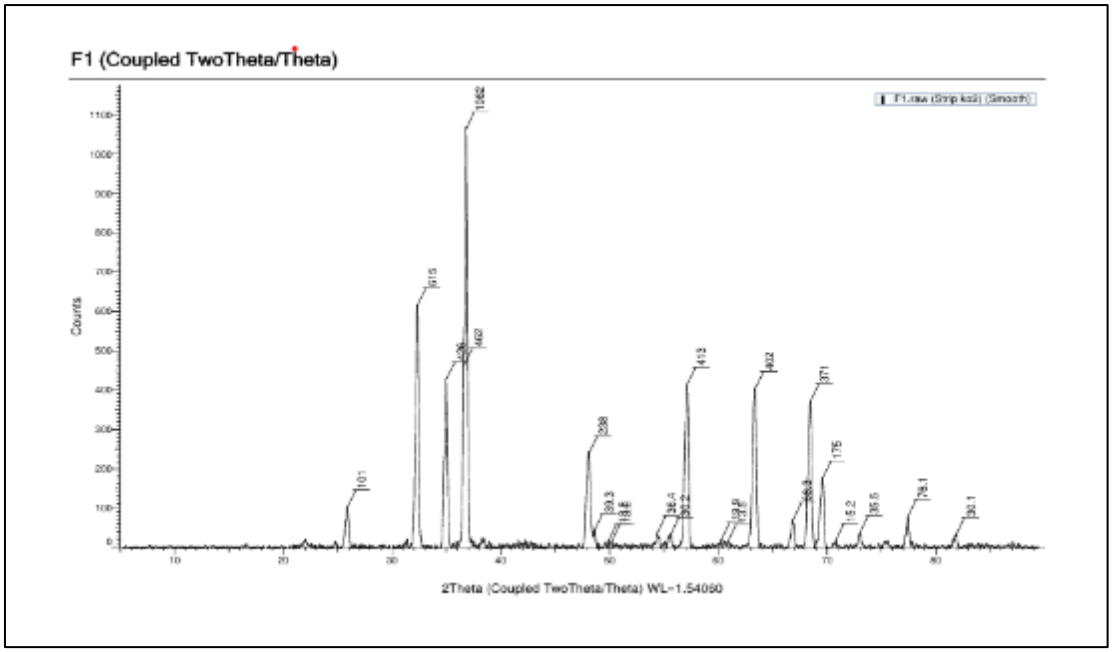

Figure 10 XRD pattern of sunscreen formulation F1

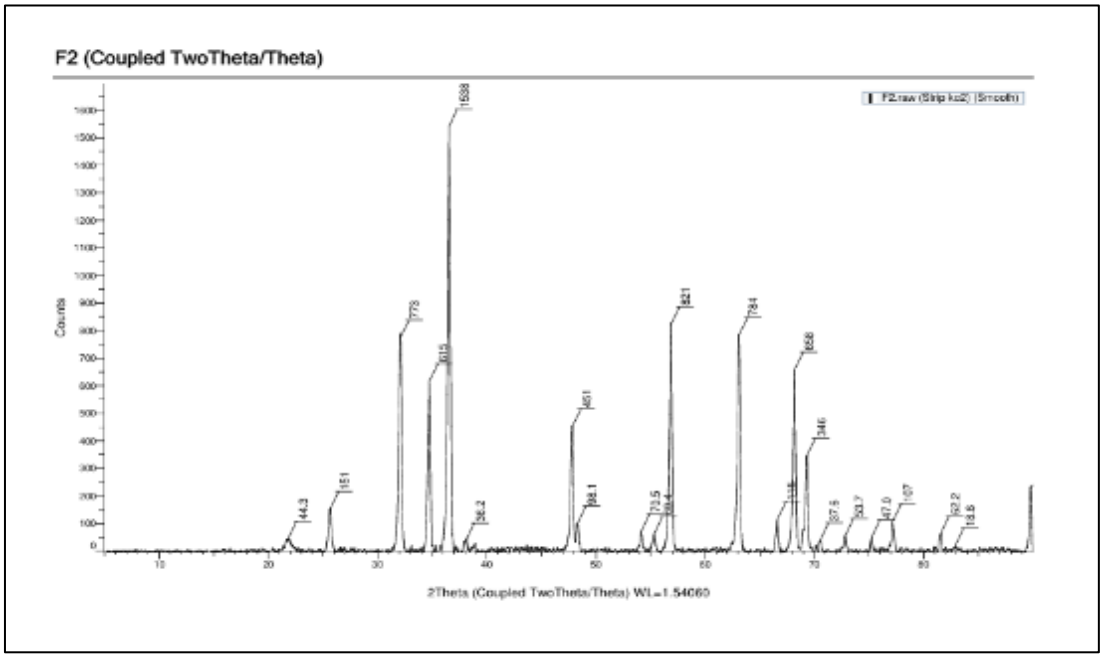

Figure 11 XRD pattern of sunscreen formulation F2

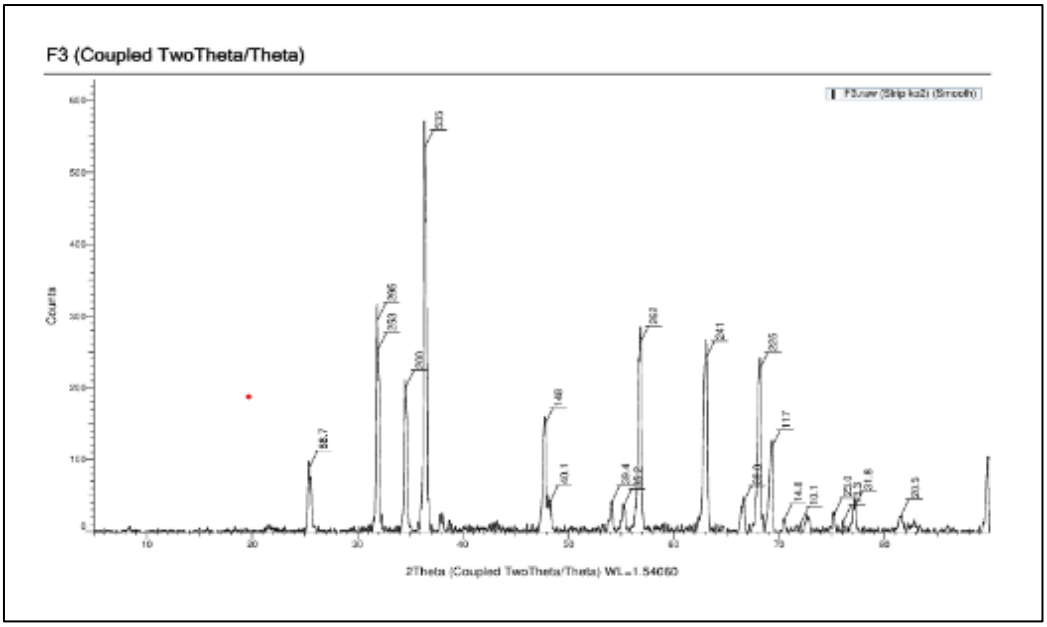

Figure 12 XRD pattern of sunscreen formulation F3

Table 16 Comparative XRD analysis of formulations F1, F2 and F3

Formulation	Nature	Observation
F1	Semi-crystalline	Broad peaks, reduced intensity
F2	Semi-crystalline	Slightly sharper peaks
F3	Semi-crystalline	Moderate peak intensity
Overall	—	No new peaks, no interaction

4.7. Determination of sun protection factor (SPF) by Mansur method

The Sun Protection Factor (SPF) of the sunscreen formulations was analysed by the use of UV spectrophotometry. The SPF was calculated by the Mansur equation. A stock solution was prepared by dissolving one gram of each formulation in ethanol. The solution was then diluted to obtain the desired concentration. The solutions were diluted twice before the actual measurements to ensure that the absorbance falls within the linear range of the spectrophotometer. The solutions were then filtered, and the absorbance was recorded over the wavelength range of 290-320 nm.

Table 17 Absorbance Values of Formulations

Wavelength (nm)	F1	F2	F3
290	0.977	1.182	1.262
295	0.945	1.150	1.222
300	0.902	1.117	1.177
305	0.931	1.106	1.168
310	0.837	1.156	1.227
315	1.030	1.266	1.364
320	1.171	1.320	1.548

Table 18 Final SPF Values

Formulation	SPF (Measured)	Dilution Factor	SPF (Final)
F1	9.19	3	27.57
F2	11.40	3	34.20
F3	12.10	3	36.30

The SPF value of the formulations F1, F2, and F3 was calculated using the Mansur equation, which resulted in the SPF value of 9.19, 11.40, and 12.10, respectively. After the application of the dilution factor of 3, the final SPF value of the formulations resulted in 27.57, 34.30, and 36.30, respectively. Among the formulations, the highest SPF value was observed in formulation F3, indicating the better absorption of UV rays. Similarly, the SPF value of formulation F2 was also observed to be comparable. In contrast, the SPF value of formulation F1 was observed to be lower.

The above results clearly indicate that the formulations have the capability to provide good to high protection. The increase in the SPF value of the formulations is due to the improvement in the absorbance of the UV rays in the UVB range of 290-305 nm, which is of major importance in the role in SPF determination.

5. Conclusion

The specific objectives of the present study are the formulation and evaluation of chitosan-encapsulated spirulina sunscreen creams (F1, F2, and F3). Preliminary phytochemical screening of spirulina was performed, and it was confirmed that the herbal drug contained important bioactive constituents such as carbohydrates, proteins, phenolic compounds, flavonoids, and saponins, which are reported to possess antioxidant and photoprotective properties. Physicochemical evaluation of the herbal drug was performed, and it was confirmed that the values of total ash, acid-insoluble ash, moisture content, and extractive values of the herbal drug are within the limits, indicating its purity and suitability for incorporation into topical formulations.

The prepared sunscreen formulations were subjected to different physicochemical evaluations, which include the assessment of the organoleptic characteristics, pH, spreadability, viscosity, homogeneity, and type of emulsion. From the results obtained, it was evident that all the formulated sunscreens showed a smooth texture, uniform appearance, and odour. This is a desirable property for a sunscreen formulation. In addition, the pH values obtained for the formulated sunscreens were in the range of 6.22 to 6.59. This indicates that the formulated sunscreens will not cause skin irritation. The spreadability and viscosity values obtained were high, which is a desirable property. Homogeneity tests showed the absence of lumps and phase separation. In addition, the dilution test showed that all the formulated sunscreens were oil-in-water emulsions.

From the in vitro antioxidant activity test carried out using the DPPH and FRAP methods, it was evident that all the formulated sunscreens showed free radical scavenging activity. Among the formulated sunscreens, F3 showed the highest antioxidant activity compared to F2 and F1. This may be due to the presence of bioactive compounds in spirulina, which help in protecting the skin from oxidative stress caused by UV radiation.

Fourier-transform infrared (FTIR) spectroscopy was employed to analyse the formulations, revealing the presence of characteristic bands associated with diverse functional groups, including O-H, C-H, C=O, and C-O. The absence of any alterations or disappearances of these bands suggests that no chemical interactions occurred among the formulation components. XRD analysis of the formulations indicated semi-crystalline nature with amorphous characteristics, indicating good dispersibility of the ingredients in the formulation, thereby contributing stability.

The Sun Protection Factor (SPF) of the formulations was performed by employing UV spectrophotometry, while the SPF values were calculated by employing the Mansur method. As a result of applying a three-fold dilution factor, the SPF values of the formulations were found to be 27.57, 34.30, and 36.30 for F1, F2, and F3, respectively. Out of the formulations, F3 was found to possess the highest SPF value, indicating better absorption of UV rays by the formulation, while UV rays are important in sun protection. F2 also showed comparable SPF values, while F1 showed relatively low SPF values.

Therefore, the study was successful in developing stable and effective herbal sunscreen formulations, which exhibit good physicochemical properties, antioxidant activities, and good to high sun protection. The study concluded that

spirulina may be regarded as a potential herbal ingredient to improve the sun protection efficacy of the sunscreen formulation. It is recommended to optimize the formulation and to conduct in vivo studies to improve the SPF values.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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