IJBPAS, March, 2024, 13(3): 1046-1054

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

FORMULATION AND EVALUATION OF TOPICAL ANTI-ACNE GEL USING *SPIRULINA PLATENSIS*

LUTE S*, WATEKAR A, SHENDE A AND KHARWADE R

Dadasaheb Balpande College of Pharmacy, Besa, Nagpur, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, (MS), India

***Corresponding Author: Ms. Shivani Lute: E Mail: slute5720@gmail.com**

Received 4th Feb. 2023; Revised 15th March 2023; Accepted 24th May 2023; Available online 1st March 2024

https://doi.org/10.31032/IJBPAS/2024/13.3.7375

ABSTRACT

Acne is a persistent skin problem that happens when dead skin cells obstruct hair follicles. The various types include spots, blackheads, whiteheads, inflammations, redness, or oily skin. There are two types of acne: inflammatory and non-inflammatory. The main objective of this research was to formulate and evaluate topical gel using *Spirulina platensis*, Vitamin C and Vitamin E. The method used for gel preparation was by extracting spirulina extract by using sonication process and further purified by dialysis method. The topical gel was prepared using extract of *Spirulina platensis* along with Vitamin C and Vitamin E. The prepared gel was kept at room temperature for 24 hours and the further evaluations were carried out. The result showed that spirulina has good anti-acne, antioxidant and anti-wrinkle property. It was concluded from study that the formulation with concentration 0.2% *Spirulina platensis* was appropriate formulation for topical treatment of acne and wrinkles.

Keywords: *Spirulina platensis*, anti-acne, anti-aging, anti-wrinkle, antioxidant

1. INTRODUCTION

Skin diseases typically develop as a result of blood pollutants, poor eating habits, and unhealthy lifestyle choices accumulating poisons in the blood. The majority of adolescents get acne vulgaris during puberty because of hormonal changes that alter pathophysiologic variables. This disorder has comedones that are open or closed, inflammatory papules, and nodules associated with it. It is also associated with blockage in follicular distention. Acne is a skin disorder that is associated with gram-

positive bacteria like Staphylococcus, Propionibacterium, and Escherichia species. The face, upper chest, and back are examples of skin regions with dense sebaceous follicles that are typically affected by acne vulgaris. An acute acne vulgaris symptom includes pain, tenderness or erythema **[1, 2]**.

One type of spirulina, *Arthrospira platensis*, is a microscopic filamentous marine cyanobacterium that is used as a food supplement and contains 70% protein by weight. The phycobiliproteins (proteins that resemble antennas and are involved in light harvesting) allophycocyanin, C-PC, and phycoerythrin are among these proteins. Holoprotein C-PC is also referred to as phycobiliproteins. It is in charge of the majority of the natural advantages that spirulina offers. Strong free radical scavenging action, inhibition of the creation of pro-inflammatory cytokines like TNF, suppression of cyclooxygenase-2 (COX-2) expression, and a reduction in prostaglandin E2 production are all properties of C-PC and -carotene. Topical acne treatments are in the lead because they are effective, patient-friendly, safe, diluted, affordable, and accessible. Spirulina is hence natural and has anti-inflammatory and antioxidant qualities **[4]**.

2. MATERIAL AND METHODS

2.1 Material

Spirulina platensis powder was obtained from Genera Nutrients Pvt. Ltd. Tamil Nadu, India. Propylene glycol-400, ethanol, carbopol-940, methylparaben, propylparaben, ethylene diamine tetra acetate [EDTA], triethanolamine were obtained from Loba Chemie, Mumbai.

2.2 Extraction and Purification of Spirulina Extract

There are two ways used for isolation of C-PC i.e. cold maceration and sonication. In sonication process, 1:25 (w/v) of dried Spirulina powder in water was allowed to sonicate at 40 kHz for 45 min. The semi liquid mixture was obtained which was further centrifuged at 1000 rpm for 20 min at 4°C. From the product obtained, the precipitate was discarded and the useful supernatant layer was collected for further use. For forgoing stages, the pH was set to pH 7.0 **[4]**.

The purification of the extract can be done by dialysis and gel filtration. The solution was dialyzed for one liter of 0.005 M sodium phosphate buffer overnight at 4 °C (pH-7.0). By briefly passing the resulting solution over a Sephadex G-25 column (12×2 cm), the solution was filtered. The flow rate used to gather the elements was 0.5 ml/min **[4]**.

2.3 Antibacterial Activity of Spirulina Extract

Antibacterial activity of extract was performed by agar well diffusion method. By suspending a 24-hour fresh culture of the bacterium *S. aureus* and *S. epidermis* in numerous 15mL of sterile water, a uniform suspension of microorganisms was obtained **[6]**.

20 mL of liquefied agar media that had been previously injected with 0.1 mL of bacteria was transferred into a sterile petri dish with an internal diameter of 8.5 cm, and the medium was left to develop a homogeneous thickness in the petri dish. A cork borer with a 6 mm diameter was used to create the wells aseptically after the liquid inoculation media had fully solidified. The wells on the plates were carefully filled with 100mg/mL of each extract, and the extracts were allowed to pre-diffuse for 30 min. The petri plates underwent pre-diffusion and were incubated at 37°C for 24 hours in the incubator before the zone of inhibition for its antibacterial activity was evaluated **[6, 7]**.

2.4 Method of Preparation of Gel

Different batches of topical gel were prepared using spirulina extract. All batches were prepared by using Carbapol 940, propylene glycol-400, methylparaben, propylparaben ethanol, EDTA, triethanolamine and quantity sufficient of distilled water to prepare 50 g of gel. Required amount of water for the formulation was divided in two parts. In first part, precise amount of extracts were dissolved separately in 15 ml of water and to this measured quantity of propylene glycol-400 and ethanol were added to it. In second part, calculated amount on Carbapol-940 was dissolved in 35 ml of water. To the above solution methylparaben, propylparaben and EDTA (Ethylenediaminetetraacetic acid) were added. Both parts one and two were mixed together in a beaker and drop wise triethanolamine was added to the formulation for adjustment of skin pH (6.8-7) and to obtain required consistency of gel. The prepared gels were stirred using propeller for 2 hours at 500 rpm. After stirring, the gel appeared to be homogeneous and devoid of any bubbles and was kept at room temperature for 24 hours as shown in **Table1 [8]**.

Table 1: Formulation of topical gel with different concentration of herbal extract

S. No.	Ingredients	F1	F2	F3
		Step first		
1	Spirulina extract	0.1%	0.2%	0.3%
2	Vitamin C	0.1%	0.1%	0.1%
3	Vitamin E	0.1%	0.1%	0.1%
4	Propylene glycol-400	4%	4%	4%
5	Ethanol	3%	3%	3%
6	Water	15ml	15ml	15ml

Step second				
5	Carbapol-940	1%	1%	1%
6	Water	35ml	35ml	35ml
7	Methylparaben	0.2%	0.2%	0.2%
8	Propylparaben	0.2%	0.2%	0.2%
9	EDTA	0.03%	0.03%	0.03%
10	Triethanolamine	0.025%	0.025%	0.025%

2.5 Physicochemical Evaluation of Gel

2.5.1 Physical parameters

Physical appearance - The physical appearance of the formulation was checked by visually by looking after the color against white board. Consistency - It was checked by applying formulation on the skin surface. Greasiness – It was checked by applying on the skin surface. Odor – The odor was checked by mixing small amount of gel in water and by taking the smell.

2.5.2 pH

pH was checked by diluting 10 g of formulation and measured using digital pH meter at constant temperature within 24 hrs. of batch preparation.

2.5.3 Viscosity

Cone and Plate Viscometer was used to determine the viscosity of the batches formed. In this, a definite quantity of gel was added to the beaker covered with a thermostatic jacket. The gel was then rotated at 100 rpm and spindle number 7 was used **[9]**.

2.5.4 Spreadability

Two sets of a glass slide with standard dimension were taken. Formulated gel was placed in between the two slides and sandwiched about the length of 60mm. Removed the adhered excess gel on the surface of the glass slides and fix to a stand without any disturbance. In the upper slide, 20 g weight was tied and noted the time taken for movement of the upper slide to the distance of 60mm under the influence of weight.18 Mean time was calculated by repeating the experiment three times and the spreadability was calculated by using

Spreadability = (m*l)/t (Equation 1)

Where m=weight tied to upper slide, l= length of the glass slide, t=time in seconds.

2.5.5 Antibacterial activity studies

20 mL of liquefied agar media that had been previously injected with 0.1 mL of bacteria was transferred into a sterile petri dish with an internal diameter of 8.5 cm, and the medium was left to develop a homogeneous thickness in the petri dish. A cork borer with a 6 mm diameter was used to create the wells aseptically after the liquid inoculation media had fully solidified. In sterile test tubes, 500 mg doses of gel were weighed and diluted with 2 mL of sterile water. The zones of inhibition were evaluated after the drug

solution had been carefully placed into the cup and had been incubated at 37 °C for 24 hours **[6]**.

2.5.6 Accelerated stability studies

The studies were performed by storing the gel formulation in sealed glass vial and kept at 40±2°C at 75±5 % for 1 month and then sample was analyzed for its physicochemical analysis, pH, viscosity etc. **[3]**.

2.5.7 Drug content

Dissolve 1g of gel in 100 ml of solvent (phosphate buffer pH 6.8 + ethanol in ration 40:60). Later keep the solutions for 4 hrs. and after that shake then and again keep for 6 hrs. for complete dissolution of the formulation in solvent. After proper dissolution the solution was filtered through 0.45mm membrane filters and proper dilutions were made, and the solution was subjected to the spectrophotometric analysis. The drug content was calculated by the linear regression equation which was obtained from the calibration data **[5]**.

2.5.8 Antioxidant activity of extract

Utilizing the 2, 2 diphenyl 1-picralhydraziyl free radical, antioxidant scavenging activity was investigated (DPPH). Making a 0.2 mM methanolic DPPH solution required adding 7.8 mg of DPPH to 100 ml of water.

Preparation of extract solution: 10 mg of *Spirulina platensis* extract was added in 10 ml and then sonicated for 15 min at 50°C.

From the above solution various dilution were prepared and made up to 10ml. 1.5 ml of various dilutions of the test material was mixed with 1.5ml of 0.2mM methanolic solution. After an incubation period of 30 min at 25°C, the absorbance at 520nm, the wavelength of maximum absorbance was recorded as A (sample). A blank experiment was carried out applying the same procedure to a solution without test material and absorbance was recorded as A (blank). Standard ascorbic acid was taken.

The free radical scavenging activity of each solution was then calculated as percent inhibition according to the equation:

$$\% \text{ inhibition} = \frac{A(\text{blank}) - A(\text{sample})}{A(\text{blank})} \times 100$$

Antioxidant activities of extracts were expressed as IC_{50} defined as the concentration of test material required to cause 50% decreases in DPPH concentration **[4]**.

3. RESULTS

In total three formulations were prepared of spirulina extract. The gelling agent Carbopol 940 was used.

3.1 Physicochemical evaluation of gel formulation:

The gel compositions' color, homogeneity, phase separation, and consistency were all

visually assessed **(Table 2)**. The formulation had consistent color dispersion, no lumps, was devoid of any particles, and was simple to wash. With a pH of 6.8, a viscosity of 6705cps at room temperature, and a spreading coefficient of 12.05g cm/sec, the F2 formulation has the highest compatibility with skin of all the formulations. It also has the best viscosity and spreadability **(Table 3)**.

3.2 Antibacterial activity of extract

Studies have shown that *Spirulina platensis* powder has antibacterial action against the *Staphylococcus aureus* and *Staphylococcus epidermis* bacteria that cause acne. The activity against *Staphylococcus aureus* was 10±0.20 mm, and the activity against *Staphylococcus epidermis* was 11±0.08 mm, according to the data.

3.3 Antibacterial activity of gel

By measuring the zone of inhibition (in mm), the well diffusion method was used to conduct antibacterial activity tests. The formulated gel study findings demonstrated dose-dependent antibacterial effectiveness against the acne-causing bacteria. **Figure 1** displays the antibacterial activity study of the formulation. The formulated gel F2 has greater antibacterial activity when compared to all other formulations, with zones of inhibition of 39 mm for *Staphylococcus aureus* and 48 mm for *Staphylococcus epidermis*. Additionally, when compared to individual extracts, the gels demonstrated a synergistic effect that may be helpful in the treatment of local inflammation. Therefore, this topical formulation is appropriate for the treatment of localized acne and was chosen for further testing.

3.4 Accelerated stability studies

On storage of spirulina gel sample at 40±2°C at 75±5%, the formulation appeared to be clear with not any variation in pH, spreading coefficient and viscosity as shown in **Table 5**.

3.5 Antioxidant activity

Antioxidant activity of Spirulina platensis cream formulation was obtained by DPPH radical scavenging activity and by considering ascorbic acid as standard. F2 shows maximum %inhibition of DPPH which was about 70% as shown in **Figure 2**.

Table 2: Physical appearance of formulated Spirulina gel formulation

Sr. No.	Formulation	Color	Homogeneity	Phase Separation	Consistency
1	F1	Dark green	Uniform	None	Excellent
2	F2	Dark green	Uniform	None	Excellent
3	F3	Dark green	Uniform	None	Excellent

Table 3: Physicochemical evaluation of Spirulina gel formulations

Sr. No.	Formulation	pH	Viscosity(cps)	Spreading Coefficient (g cm/sec)
1	F1	6.2±0.06	6650±10	11.57±0.25
2	F2	6.8±0.05	6705±95	12.05±0.56
3	F3	6.6±0.03	6554±70	9.57±0.91

Table 4: Drug Content of the formulation

Formulation	Drug Content (% m/m)
F1	92.4
F2	96.3
F3	94.7

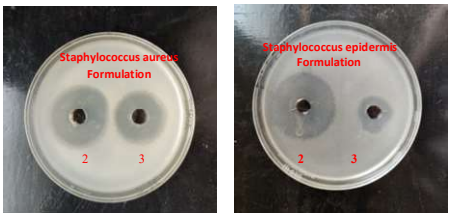

Figure 1: Antimicrobial activity of formulations

Table 5: Accelerated stability study result of spirulina formulation F2

Sr. No.	Parameters	Result
1	Appearance	Clear
2	Color	Dark green
3	pH	6.8±0.15
4	Spreading coefficient	12.02±0.87 g.cm/sec
5	Viscosity	6250cps

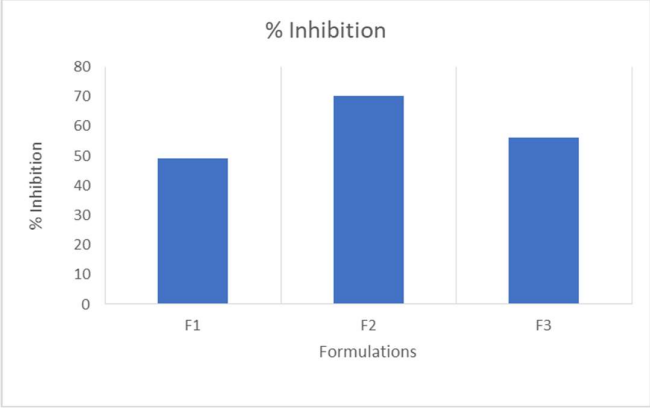

Figure 2: Antioxidant activity of formulations

4. DISCUSSION

About 80% of adolescents experience acne vulgaris, a long-term inflammatory skin condition, during the puberty stage. This skin ailment has been associated to gram-positive bacteria like *Staphylococcus, Propionibacterium, and Escherichia* species. The use of antibiotics to treat this issue more frequently explores a number of negative effects. As a result, the herbal formulation must be the primary focus of treatment **[12]**.

There are many potential phytoconstituents present in spirulina which can be used as antioxidant, anti-acne, anti –inflammatory, antibacterial, also used in brightening of skin and in wound healing.

From the antibacterial activity studies it was observed that the extract of spirulina shows exclusive antibacterial activity against gram positive bacteria i.e.

Staphylococcus aureus and *Staphylococcus epidermis* with diameter of zone of inhibition 10±0.20mm and 11±0.08 mm. Physicochemical studies showed that formulation F2 has pH compatible to skin i.e. 6.8, with viscosity 6250 cps at room temperature and spreading coefficient of 12.02g cm/sec. Antibacterial activity of the formulation showed that formulation F2 has zone of inhibition of 39mm for *Staphylococcus aureus* and 48 mm for *Staphylococcus epidermis*. The formulated gel showed synergistic activity along with vitamin C and vitamin E. Because it caused no irritation, this topical formulation was acceptable for the treatment of localised acne and was chosen for further testing.

5. CONCLUSION

In recent years, herbal remedies for acne vulgaris are increasingly frequently regarded as secure and having fewer adverse effects than synthetic medications. Consequently, in the world market there is a high demand for herbal formulations and other natural medicines. The formulation and evaluation of the anti-acne gel as well as the stability studies represent extremely good efforts. Based on the studies, the herbal gel prepared from extract of *Spirulina platensis* has shown great antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermis* without any significant effect on skin. When compared to the extract, the gel had a synergistic impact and was more stable. The study's findings therefore suggest that the gel formulation containing 0.2% of an extract of *Spirulina platensis* can be used to treat acne vulgaris.

6. REFERENCES

[1] Pachpawar, N.G., Mahajan, U.N., Kharwade, R.S. Formulation and Evaluation of sun protective topical preparation. International Research Journal of Pharmacy, 9 (2), 2018, 27-32.

[2] Mate, A., Ade P., Pise, A., More, S., Pise, S., Kharwade, R. Formulation and Evaluation of polyherbal gel for the management of acne. International Journal of Current Research and Review, 13 (4), 2021, 117-122.

[3] Kharwade, R.S., Mahajan, N.M. Formulation and Evaluation of Nanostructured Lipid Carriers Based Anti-inflammatory gel for topical drug delivery system, 12 (4), 2019, 286-291.

[4] Badduri, N., Gupta, N.V., Gowda, D.V., Manohar, M. Formulation and Evaluation of topical anti acne formulation of spirulina extract. International Journal of Applied Pharmaceutics, 10(6), 2018 229-233.

[5] Agnihotri, S., Wakode, S., Agnihotri, A. Formulation and

Evaluation of herbal anti acne gel of *Myrica Esculeta*. Asian Journal of Pharmaceutical and Clinical Research, 9 (4), 2016, 358-361.

[6] Bhinge, S.D., Bhutkar, M.A., Randive, D.S., Wadkar, G.H., Kamble, S.Y., Kalel, P.D., et al. Formulation and Evaluation of polyherbal gel containing extracts of Azadirachta indica, Adhatoda vasica, Piper betle, Ocimum tenuiflorum and Pongamia pinnata. Marmara Pharmaceutical Journal, 23(1), 2019, 44–54.

[7] Singh, S. Extraction and characterization of Orange peel. World Journal of Pharmaceutical Research, 2015, 20.

[8] Bhutkar, K. Formulation and Evaluation of herbal. 11, 2019, 291-295.

[9] Misal, G., Dixit, G., Gulkari, V. Formulation and Evaluation of herbal gel. Indian Journal of Natural Products and Resources, 3(4), 12, 501–505.

[10] Gatne, M.M., Tambe, K., Adarsh, Ravikanth, K. Acute Dermal Irritation study of polyherbal gel Mastilep in rabbits. International Journal of Pharmaceutical Science and Research, 6(8), 2015, 3473–3476.

[11] Zhang X, Liu J, Liu H, Ni J, Zhang W, Qiao H, *et al*. Formulation and optimization of dihydroartemisinin nanostructured lipid carries using response surface methodology. Powder Tech 2010; 197:120-8.

[12] Suva, M.A., Patel, A.M., Sharma, N. A Brief Review on Acne Vulgaris: Pathogenesis, Diagnosis and Treatment. Res Rev J Pharmacology, 4(1), 2016:1–12.

[13] Prasad SB. Acne vulgaris: A review on pathophysiology and treatment. Asian Journal of Pharmaceutical and Clinical Research, 9(4), 2016; 9(4):54–59.