

Formulation and evaluation of antiwrinkling moisturizing cream enriched with *Ulva reticulata* extract

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Abstract: Skin ageing is a natural process occurs by environmental factors, oxidative damage and loss of moisture content which leads to the formation of wrinkles. There is a demand for herbal based skincare products have increased due to their reduced risk of side effects. *Ulva reticulata* is a marine macro alga highly known for its antioxidant and hydrating properties. The moisturizing cream was developed by incorporating *Ulva reticulata* which is the active ingredient. Physiochemical parameters such as pH, spreadability and stability testing were performed to ensure the optimal formulation quality. Antioxidant assays confirmed the formulated cream has the ability to neutralize free radicals which plays a main role in wrinkle formation. The formulated moisturizing cream were assessed with their phytochemical property, antimicrobial property, spreadability and antiwrinkling property. This study highlights the promising rope of *Ulva reticulata* in developing natural, functional cosmetics. The result demonstrated that formulated moisturizing cream has good pH, spreadability and there is no phase separation in the cream.

Key Words: *Ulva reticulata*, antioxidant, anti- inflammatory, phytochemical, spreadability.

1. INTRODUCTION

Ulva reticulata commonly known as reticulated seaweed, it is a green algae belonging to *Ulvaceae* family. It stands out for its unique net like, perforated blade structure often forming ribbon like formations with irregularly shaped holes. This seaweed thrives in warm marine environments to rocky surfaces. However, as it matures, parts of it can break off and float freely in the water. Beyond its ecological role, *Ulva reticulata* is packed with beneficial nutrients, including proteins, vitamins, and sulfated polysaccharides. These compounds are known for their antioxidant and anti-inflammatory properties, making this seaweed a valuable ingredient in skincare. Its antioxidants help neutralize free radicals, protecting the skin from oxidative stress and signs of aging. Additionally, its anti-inflammatory effects can soothe irritation and reduce redness, making it ideal for sensitive or inflamed skin ¹.

Commonly, the phytochemicals in seaweeds belong to the chemical classes including brominated phenols, oxygen heterocyclics, nitrogen heterocyclics, sulfur-nitrogen heterocyclics, sterols, terpenoids, polysaccharides, peptides, proteins, halogenated ketone, alkanes, and cyclic polysulfides ². Moreover, *Ulva reticulata* contains natural antimicrobial compounds, which may help promote skin health and resilience by protecting against harmful microorganisms. These properties make it a promising addition to moisturizing cream, enhancing hydration while offering protective and soothing benefits³.

2. METHODOLOGY

Collection and extraction of samples

The *Ulva reticulata* was collected from Park Bay in Rameswaram district. The algae samples were dried and grinded into fine powder. The extraction was done by Soxhlet in an aqueous solution.

Qualitative analysis of phytochemical screening:

The phytochemical analysis was carried out for the algae sample. The color change was observed for the various phytochemical compounds such as Alkaloids, Flavonoids, Saponins, Tannins, Phenol, and Steroids.

Antimicrobial activity for the *Ulva reticulata* extract

The antimicrobial activity was carried out for the sample by using the test culture *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas* sp, *Klebsiella* sp, and *Candida albicans*. The nutrient agar plates were prepared,

and the test organisms swabbed on it. Then add the sample into the well in different concentrations (25 μ l, 50 μ l, 75 μ l and 100 μ l). Then placed in an incubator for 24-48 hours at 37 °c. The zone of inhibition was observed and recorded.

UV for *Ulva reticulata* extract

Different colored pigments vary in the wavelength of light that they absorb. Most pigments are conjugated compounds with alternating double and single bonds and typically absorb light in the visible region. The conjugated part of the dye molecule can be very short, meaning that there is a low degree of conjugation and few alternating double and single bonds, or long. Meaning that there is a high degree of conjugation with many alternating double and single bonds.

The extracts were examined under the visible UV -visible spectrum. The extracts were scanned in the wavelength ranging from 294nm using systronic spectrophotometer. The blank was kept added to the cuvette and the readings were taken for the absorbance. The peak value of the UV visible was recorded ⁴.

FTIR spectroscopy for *Ulva reticulata* extract

Fourier transform infrared spectroscopy (FTIR – 4600 TYPE A). Turn on FTIR, a wait for 3 characteristic beeps from the instrument. Ensure the instrument is calibrated and run a background scan. Clean the sample holder, apply one drop or a small micro spatula portion (15-20 mg) to fill the dwell 50% full. Make sure not to scratch the crystal with spatula or any other metal. Apply the press to the sample, screw it up. Select monitor sample from the measure menu and read the spectra. Save the spectra, power off the equipment after measurement⁵.

Product formulation

The moisturizing cream was prepared by using *Ulva reticulata* extract along with beeswax, coconut oil, potassium sorbate liquid paraffin, gelatin and glycerin. Initially, beeswax (30gm) and coconut oil (10ml) were melted together using gentle heat to form oil phase. Simultaneously, gelatine (4gm) and glycerine (2ml) were dissolved in water to create aqueous phase. Once both the phases reached a uniform consistency, they were combined with continuous stirring to ensure emulsification. Liquid paraffin (2ml) was then added to enhance the cream texture and spread ability. Potassium sorbate (2gm) was incorporated as preservative to prevent microbial growth. *Ulva reticulata* (4ml) extract was carefully mixed into the formulation and the cream was allowed to cool, forming a smooth and stable emulsion. The prepared moisturizing cream was then stored in a container for further evaluation⁶.

Protein denaturation activity for formulated moisturizing cream

In a set of test tubes 0.1ml to 1 ml of Bovine serum albumin solution (standard protein) and 4.78 ml of phosphate buffer solution was added which was kept for standard. In other test tubes 0.1 ml of bovine serum albumin and 4.78 ml of phosphate buffer was added along with formulated moisturizing cream. The test tubes were kept for incubation at 37° c for 15 minutes in water bath and subjected to incubation 70° c for 5 minutes. The turbidity is measured at 620 nm using a calorimeter after the sample is cooled.

Anti oxidant activity for formulated moisturizing cream

The anti-oxidant activity is that molecule can neutralize free radicals by donating an electron or hydrogen atom, effectively stabilizing free radicals and preventing further oxidative damage to biological molecules like proteins, lipids, and DNA: this process is often achieved by various assays. Antioxidant activity was carried out by (DPPH) assay using a modified method Brand Williams. 5 test tubes were taken and 100 to 500 l of sample was added. Then DPPH solution was added to all the tubes. The mixture was shaken vigorously and kept at room temperature for 30 minutes. The reaction was measured at 560 nm using calorimeter⁷.

3. RESULT

Collection and extraction of sample:

The *Ulva reticulata* sample was collected from Rameshwaram district and extracted by using Soxhlet in an aqueous solution.

Figure 1: Extract of *Ulva reticulata*



Phytochemical analysis for the *Ulva reticulata* extract:

The phytochemical screening of the extract showed the presence of tannins, alkaloids, saponins, and proteins. However, compounds such as steroids, terpenoids, glycosides, phenols, quinones, carbohydrates, flavonoids, and iodine alkaloids were not detected. The presence of tannins indicates potential antioxidant and antimicrobial properties in the extract. Tannins are known to help combat oxidative stress and may contribute to the preservation and protection of skin tissue. Alkaloids, another key group of bioactive compounds found in the extract, are well recognized for their anti-inflammatory and antimicrobial effects. These properties make them particularly valuable in wound healing and skincare applications. Green seaweeds are also rich in polyphenols like catechin, epicatechin, gallate, and gallic acid. According to a study by phenolic compounds were identified in the chloroform extract of green seaweed⁸. These compounds are known for their strong antioxidant activity, which helps fight disease caused by free radicals. Additionally, phenolic compounds are widely utilized for their anti-inflammatory, antimicrobial, antiviral, and anticancer benefits. (Table 1)

Table 1: Phytochemical analysis of *Ulva reticulata* extract

S.NO	TEST	RESULT
1	Tannins	+
2	Alkaloids	+
3	Saponins	+
4	Steroids	-
5	Terpenoids	-
6	Glycoside	-
7	Phenol	-
8	Quinone	-
9	Proteins	+
10	Carbohydrates	-
11	Flavonoids	-
12	Iodine alkaloids	-

Table 2: Product formulation (Moisturizing cream)

S.NO	MATERIALS	FUNCTION
1	Bee wax (30gm)	Hydrate and soothing
2	Coconut oil (10ml)	Retain moisture
3	Potassium sorbate (2gm)	Preservative
4	Liquid paraffin (2ml)	Emollient, protective barrier
5	Gelatin (4gm)	Thickening agent
6	Glycerin (2ml)	Humectants
7	<i>Ulva reticulata</i> extract	Antioxidant activity
8	Lolita oil (2 drops)	Fragrance

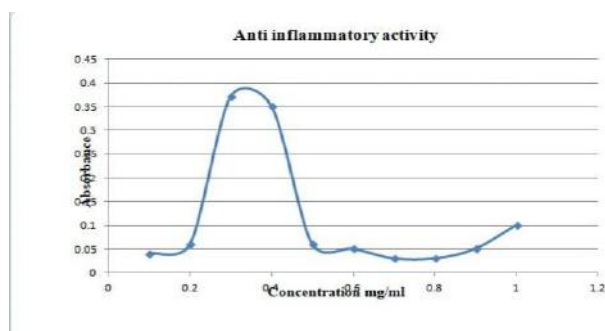
Figure: 2 Product formulation (Moisturizing cream)



Analysis of protein denaturation for moisturizing cream

Protein denaturation method assayed the anti- activity for moisturizing cream. The graph indicates that formulated moisturizing cream may exert its anti-inflammatory effects by preventing protein denaturation a key process in anti-inflammatory responses. (Figure 3)

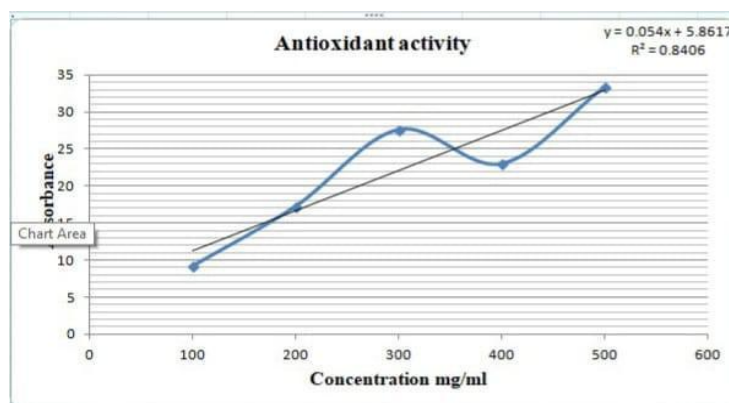
Figure 3: Anti-inflammatory activity for moisturizing cream



Antioxidant activity for formulated moisturizing cream

The DPPH method assayed the antioxidant activity for moisturizing cream. DPPH Scavenged (%) = (absorbance of control-absorbance of test) × 100/control of absorbance. The antioxidant activity of the extract was evaluated using the stable DPPH (2,2-diphenyl -1-picrlhydrazyl) free radical method. A volume of 400 µl of the prepared extract was mixed with 0.1ml DPPH solution in methanol, and the final volume was adjusted to 2.0 ml. The mixture was then incubated for 30 minutes at room temperature, after which the absorbance was measured at 580nm. The percentage of DPPH radical scavenging was calculated by comparing the absorbance of the sample with that of control. The antioxidant activity of the extract was then determined based on the quercetin standard calibration curve, as described (Figure 4)

Figure 4: Antioxidant activity for moisturizing cream



UV spectroscopy for *Ulva reticulata* extract

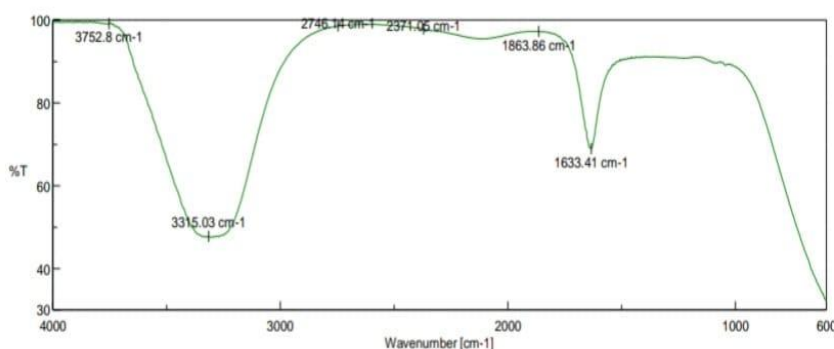
Spectroscopy method has become a powerful tool for secondary metabolite profiling as well as qualitative and quantitative analysis of pharmaceutical and biological material.

The UV spectroscopy was characterized for the *Ulva reticulata* extract. The peak was observed in the wavelength range 250nm.

FTIR spectrum analysis of *Ulva reticulata* extract

The FTIR Spectrum was used to identify the functional groups active compounds by analyzing the peak values in infrared region. FTIR Spectrum of *Ulva reticulata* attains four peak which contains bond strength of medium to strong weak, and strong bond. Peak 1 contain medium to strength bond and it belongs to the functional group of O-H it consists of Alcohol compound. In peak 2 and 3 attains a bond strength of -COOH group and contains carboxyl group. Peak 4 belongs to strong bond and functional group of C-OH contains aldehyde compound. (Figure 5)

Figure: 5 FTIR Spectrum analysis of *Ulva reticulata* extract



Antimicrobial activity for formulated moisturizing cream

Nutrient agar plates were prepared and the test culture was swabbed on a culture plate. A well drilled at the center of the media and the cream was poured into the well. The plates were incubated at 24 hours at 37°C. The antimicrobial activity of the moisturizing cream formulated with *Ulva reticulata* extract was tested against five microbial strains: *staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* sp, *klebsiella* sp, and *candida albicans*. The cream was evaluated at four different concentrations (25µl, 50µl, 75µl and 100µl). Among the tested strains *Pseudomonas* sp showed the highest susceptibility, with a zone of inhibition measuring 30mm at the highest concentrations (100µl). (Table 3)

Methanolic extract of *Ulva reticulata* exhibited strong antibacterial activity, producing a maximum inhibition zone of 40 mm at a concentration of 200 mcg against *Escherichia coli*, and *streptococcus pyogenes*. Similarly, *cladophora glomerata* was effective against *Pseudomonas aeruginosa*, while the least activity (6mm) was observed with 50 mm of *Kappaphycus alvarezii* against *staphylococcus epidermidis*. Various concentrations of seaweed extracts showed notable antibacterial activity, particularly against *Staphylococcus aureus* and *Escherichia coli*⁹.

Table 3: Antimicrobial activity for formulated moisturizing cream

S.NO	ORGANISMS	25(µl)	50(µl)	75(µl)	100(µl)
1	<i>S.aureus</i>	22mm	23mm	24mm	26mm
2	<i>E.coli</i>	20mm	22mm	24mm	25mm
3	<i>Pseudomonas</i>	17mm	25mm	27mm	30mm
4	<i>Klebsiella</i>	23mm	22mm	25mm	27mm
5	<i>Candida albicans</i>	20mm	22mm	24mm	27mm

Physical evaluation of formulated cream:

The prepared cream was kept in room temperature and the pH was checked by using pH strip and pH meter. The formulated moisturizing cream contains ph of 5.66 which was measured using ph meter. Spreadability testing was also performed for the moisturizing cream and the value of spreadability is 35.71. pH measurement is carried out by dissolving the cream in water with a ratio of 1: 3, stirring until homogeneous and allowed to stand until it settles¹⁰. The water is drawn and measured using a pH meter with the requirement for a good cream between 4.5 – 6.5. The formulated cream is carefully placed on the paper that is overlapped with transparent glass and left for a while. The area given by the preparation is calculated, and the cream is recovered with a glass that has a load equal to the amount of weight of each glass used and overwritten with a load of 50, 100 & 150 grams. Each load is left for 60 seconds because the wider the diameter, the better the spread. (Table 4)

Table 4: Physical evaluation of moisturizing cream

S.NO	PARAMETER	MOISTURIZER
1	Temperature	Room temperature
2	pH	6

3	Appearance	White colour
4	Consistency	Semi solid, smooth ,soft
5	Homogeneity	Good
6	Irritant test	Non irritable
7	Odour	Pleasant
8	Type of smear	Greasy

4. CONCLUSION:

The formulated anti-wrinkling cream incorporating *Ulva reticulata* extract demonstrated promising results in terms of stability, spreadability and skin computability. The physiochemical properties of the cream, including pH, and emulsification stability, were within acceptable ranges, indicating a well formulated product suitable for topical applications.

Further studies including in vivo testing and long-term stability testing are recommended to confirm its efficacy and shelf life.

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