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In Vitro Study of the Anti-inflammatory and Antioxidant Activity of the *Leptolyngbya* spp. Isolated from Lonar Lake

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ABSTRACT

Cyanobacteria are the photosynthetic organism which colonise in the diverse ecological niche from fresh water to extreme environment. Theyproduces variety of the biomolecules such as proteins, lipids, carbohydrates, amino acids, alkaloids, phenolics and flavonoids which possess various pharmaceutical, cosmeceutical and nutraceutical properties. The present research is based on the study of the *Leptolyngbya spp.* for antioxidant and anti-inflammatory potential. The *Leptolyngbya spp.* was isolated on bold basal medium from the extreme environment of the Lonar lake located in Maharashtra. The polyphenolic content (TPC) and flavonoids content (TFC) was assessed and it was found to be 27.33 ± 0.41 mg/equ. of tannic acid/gm and 40.076 ± mg/equ. of quercetin/gm respectively. The crude whole cell extract was studied for the antioxidant potential and anti-inflammatory activity. The antioxidant potential was studied by using free radical scavenging potential using the DPPH (2,2 -diphenyl-1-picryl-hydrazyl-hydrate) assay and ABTS (2,2'-Azino-Bis-3-Ethylbenzothiazoline -6-Sulfonic acid) assay. Methanolic extracts of the *Leptolyngbya spp.* showed 63.70 ± 0.58 % inhibition for the DPPH assay and 94.30 ± 1.83 percent inhibition for ABTS assay. It was observed that the polyphenols contribute majorly to the antioxidant activity. The *in vitro* anti-inflammatory activity of the methanolic extracts of the *Leptolyngbya spp.* was studied by using the Human red blood cells membrane stabilisation assay for the heat induced and hypotonicity induced condition. The *Leptolyngbya spp.* showed 96 ± 0.1% Red Blood cells membrane stabilisation for heat induced condition and the IC 50 value was found as 453 ± 3 µg/ml. The percent RBC stabilisation observed for the hypotonicity induced condition was 86 ± 0.7% and IC 50 values was 86 ± 5 µg/ml. The Aspirin was used as standard for the RBC membrane stabilisation assay. The study results revealed that the *Leptolyngbya spp.* has a significant antioxidant potential and also possess a significant anti-inflammatory activity. The antioxidant and anti-inflammatory potential of the *Leptolyngbya spp.* was used for the development of the hair care products such as hair nourishing cream. The hair nourishing cream formulation was prepared with 0.1 % whole cell extracts of the algal mass of *Leptolyngbya spp.* The formulation was stable for the period of6 months with respect to the physicochemical parameters.

Key words: ABTS, Anti-inflammatory, Antioxidant activity, Cyanobacteria, DPPH, *Leptolyngbya spp.*

1. INTRODUCTION

Cyanobacteria are the important diverse group of bacteria which produces variety of the natural biomolecules having economic importance and various biotechnological and pharmaceutical applications. They produce various biomolecules such as lipids, carbohydrates, proteins, phenolics, and flavonoids having antioxidant and anti-inflammatory properties. Algal biomass and metabolites from algae are having various applications in cosmeceutical, nutraceutical, and pharmaceutical industries. *Leptolyngbya* are thin filamentous cyanobacteria characterized by the narrow width of their cylindrical trichomes (0.5-3µm). *Leptolyngbya* spp. originates in diverse ecosystem such as marines, fresh water, swamps, forest, rice forest, alkaline lake, polar desert and hot deserts.[1] Algal species are used in cosmetics as skin whitening and antiwrinkle agent.

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Mycosporine like amino acids protects the skin against the UV damage and premature aging. Algae are also used as thickening agent and skin sensitizers in lotions and many other cosmetic products. Carrageenan obtained from the *Chondrus crispus* is the commercially available thickening agent.[2]

Cyanobacteria are the photoautotrophic organisms which are exposed to the high oxygen and radical stress resulting in the development of the effective protective system against free radicals and oxidative damage. The defensive system is basically due to production of the antioxidants by the algal cells such phenolic, flavonoids, lipid, carbohydrates and proteins.[3] DPPH (1,1-Diphenyl-2-picrylhydrazyl) and ABTS (2, 2′-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid) assays are used for the detection of the antioxidant activity.

The commercial availability of the cyanobacteria species is nowadays explored due to the continuous, reliable source of the natural products, including antioxidants and other metabolites having anti-inflammatory activity. Cyanobacteria also help to reduce hair loss by reducing the inflammation of hair follicle caused by the bacteria and also promotes hair growth, shine and volume.[4] In vitro anti-inflammatory activity is done by using Human Red Blood Cells membrane stabilisation in heat and hypotonicity induced conditions in presence of test extracts compared to the standard aspirin. Human red bold cell membranes are equivalent to the lysosomal membrane component. The prevention of hypotonicity and heat induced HRBC membrane lysis was estimated as the anti-inflammatory activity.

The study focus is on the isolation of the *Leptolyngbya* species and the evaluation of the antioxidant potential and anti-inflammatory property. The strain was isolated from the alkaline Lonar lake situated in the Maharashtra Buldhana district. The crude extract was studied for the antioxidant activity using DPPH radical scavenging assay and ABTS assay. The anti-inflammatory activity of the algal extracts was studied by using trypsin inhibition, HRBC membrane stabilization method in heat induced condition and at hypotonic condition. The strain is having a scope for the development of cosmetic and pharmaceutical industries. The extracts can be used in hair nourishment creams, lotions, moisturizers.

2. MATERIALS AND METHODS

2.1. Sampling and Isolation of *Leptolyngbya* Strains

The water sample was collected from the alkaline Lonar lake district Buldhana Maharashtra (19058′N & 760 30′E) in summer season (May 2013). The isolation and purification of algal isolates was done on bold basal media. The strain was cultured in bold basal media with alkaline pH 11 and salt concentration was 1.5 %. The growth conditions were maintained as 12 hrs day night cycle at room temperature for 15 days. The primary genus identification was done by using microscopy. The morphology and microscopy revealed the genus as *Leptolyngbya spp.*

2.2. Identification of the Algal Isolate

Identification of the algal isolate was done by using the 16S rRNA technique. The genomic DNA was extracted using the Cetyltrimethylammonium bromide method. The crushed algal mass was suspended in the Cetyltrimethylammonium bromide buffer and incubated at 60^{O}C for 60 min. The extraction was done in the chloroform and isoamyl alcohol (24:1) and centrifuged. The aqueous part was precipitated using chilled ethanol and centrifuged. The extracted genomic DNA was subjected to the electrophoresis on 1.5% agarose gel with ethidium bromide.[5] The PCR amplification of the 16S rRNA was done to identify the isolate. The forward primer 8F (5′AGAGTTTGATCCTGGCTCAG3′) and reverse primer 907R (3′CCGTCAATTCMTTTRAGTTT5′) was used for the study. The identification details and sequence was submitted to Genbank.[6] Molecular techniques have been used in many areas and this technique increasing the reliability of the work done. The general purpose of the study is to compare Cyanobacteria and Chlorophyta species under the culture conditions using classical classification and molecular identification. In order to achieve this aim, isolation of different clonal cultures of Cyanobacteria and Chlorophyta species from Apa Dam Lake and Beykavağı pond (Konya, Turkey).

2.3. Harvesting of Algal Mass and Extraction of Metabolites

The purified algal isolate of *Leptolyngbya* was grown in the bold basal media with alkaline pH and 1.5% salt. The exponentially grown algal mass was filtered and washed with pellet thrice with distilled water to remove media traces and salt. The freeze-dried algal biomass was ground in mortar and pestle to make fine powder. The extraction of the metabolites was done by using solvent tetrahydrofuran and methanol solvent in (2:8) proportion. The supernatant was dried at room 30°C for 24 hrs and kept at 4°C overnight. The dried metabolites were further used for the study.[7]

2.4. Phytochemical Evaluation of the *Leptolyngbya spp.*

Algae are the rich source of the proteins, phenols, flavonoids, lipid and alkaloid. The bioactivity of these

secondary metabolites is very high. They are used in the nutraceutical, pharmaceuticals and food industry also.

2.4.1. *Total Phenolic compound (TPC) of the Leptolyngbya spp.*

The polyphenolic content of the algae contributes majorly in the antioxidant activity and provides various benefits to end user as these are antiviral, antimicrobial in nature. The polyphenols reduces the negative effect of the free radical by neutralising the oxidative stress of radical or superoxide radicals.[8] The polyphenolic content of algae was estimated by using the Folin Ciocalteu method. The 10% Folin Ciocalteau reagent 0.5 ml methanolic alga extracts and incubated at room temperature for 5 min. 2% Na_2CO_3 was added to the reaction mixture to make the reaction mixture alkaline and incubated at room temperature in dark condition. The absorbance was measured at 700 nm on colorimeter Vidyut Kanad 0392. The tannic acid was used as standard for the assay expressed as mg/equ. of tannic acid/gm.[9]

2.4.2. *Total Flavonoids compound (TFC) of the Leptolyngbya spp.*[9]

Flavonoids have different biological functions such as ultraviolet protection; flavonoids scavenge the reactive oxygen species responsible for deterioration. Flavonoids activate the antioxidant enzymes and inhibit oxidation. The Flavonoids content was determined using Aluminium Chloride colorimetric method.

In this assay, aluminium chloride reacts with flavones or flavonols at C-4 keto group or C-3/C-5 hydroxyl group of flavones or flavonols to forms acid stable complexes. In case of A –or B-ring of flavonoids, aluminium chloride reacts with orthrodihydroxyl groups forms acid labile complexes with the orthrodihydroxyl groups in A –or B-ring of flavonoids. Under alkaline condition, the complex reacts with sodium nitrite to form yellow coloured product which is measured at 415 nm in calorimeter Vidyut Kanad 0392. The Quercetin 200 µg/ml was used as standard for the reaction expressed as mg/equ. of Quercetin/gm.

2.5. Antioxidant Activity of the Algal Extracts Using DPPH Assay[10]

DPPH assay is based on the free radical scavenging activity based on the electron transfer. DPPH (1,1-Diphenyl-2-picrylhydrazyl) is a stable free radial with red colour (absorbed at 517nm) in methanol. If free radicals have been scavenged, DPPHH ions will generate it's colour to yellow. This assay is used to show free radical scavenging activity of the compound.

Test samples were allowed to react with 0.15 mM solution of DPPH in methanol. 0.5 mM Ascorbic acid is used as standard in Methanol. The absorbance of reaction mixture was measured at 530 nm on colorimeter Vidyut kanad 0392. The experiment was performed in triplicate.

Antioxidant activity (Percent inhibition) = [(Ai-Ac)-At] /Ac x100

Ai – Absorbance of initial concentration of algae used for study
Ac – Absorbance of control
At – Absorbance of test

2.6. Antioxidant Activity of the Algal Extracts by ABTS Assay[11]

ABTS assay is based on the free radical transfer of the electron ions in aqueous phase. The $ABTS^+$ 2-2'-azino-bis (3-ethylbenzothiazoline-6 sulfonate) radical cations which turns to blue in its reduced form and gets colourless in presence of the antioxidant molecule.

The ABTS was prepared by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate. (1/1, V/V). The mixture was incubated at RT for allowing reaction to complete and to get stable absorbance. Kanad Vidyut 0392 instrument was used for the assay. The ABTS solution was diluted with 50% methanol to get absorbance in the range of 0.700 ± 0.05 at 700 nm. 0.5mM Vitamin C (Ascorbic acid) was used as standard. 1800 µl ABTS solution was added to 200 µl of pigment solution. The tubes were incubated at RT for 6 min. Absorbance was measured at 700 nm on Kanad Vidyut 0392 instrument. The antioxidant activity of the test sample was calculated by using following equation. The experiment was performed in triplicate.

Antioxidant activity (Percent inhibition) = [(Ai-Ac)-At] /Ac x100

Ai – Absorbance of initial concentration of algae used for study
Ac – Absorbance of control
At – Absorbance of test

2.7. Anti-inflammatory Activity of the Algal Extracts

During inflammation various Lysosomal enzymes are generated, these enzymes can create more inflammation. The anti-inflammatory drugs either destroys lysosomal enzymes or stabilises the lysosomal membranes. The RBC mimics the lysosomal membranes thus in the study of anti-inflammatory activity Human Red Blood Cells are used.[12]

2.7.1. *Membrane stabilisation Human Red Blood Cells Suspension Stabilisation*[13]

The Anti-inflammatory activity of algae extract was assessed by HRBC stabilisation method in Hypotonic solution. Blood was collected from healthy individual who is not undergoing any treatment especially NSAIDs treatment for at least 15 days. and mixed with the equal

volume of the Elsevier's solution containing 2% dextrose, 0.8 % sodium citrate, 0.05 % Citric acid and 0.42% sodium chloride. The blood was centrifuge at 13500 rpm for 15 min and packed cells were washed with iso saline (0.85 % pH7.2). The 10 % v/v stock was prepared using iso saline.[14]

2.7.1.1 Hypotonicity Induced Haemolysis

Hypotonic solution makes the RBCs unstable and causes haemolysis. The presence of anti-inflammatory agents maintains the red blood cells membrane stable. If the extract has anti-inflammatory activity the RBCs remains stable which is monitored by recording absorbance at 560 nm against blank phosphate buffer saline and aspirin was used as standard. The reaction mixture consists of the 400 µL of phosphate buffer saline, 800 µL of hyposaline (0.36%) and equal volume of HRBC suspension and test extracts. The reaction mixture was incubated at 37°C for 30 min and centrifuge at 3000 rpm for 20 min. The supernatant was collected and absorbance was recorded at 560 nm on colorimeter. The experiment was done in triplicate. IC50 values were calculated. Percent inhibition of haemolysis was calculated using following formula,

$$\text{Percent inhibition of Haemolysis} = (A_{Blank} - A_{Test}) / A_{Blank} * 100$$

Where A_{Blank} = Absorbance of blank
A_{Test} = Absorbance of Test

2.7.1.2. Heat Induced Haemolysis

This assay is based on the stabilisation of the RBC cell membrane by heat induced haemolysis. Anti-inflammatory drug makes the RBC membrane to stabilize at higher temperature. The extracts were evaluated for anti-inflammatory activity at 56°C.

The test was performed by mixing 500 µL test extract with the 500 µL of HRBC suspension and incubated at 56°C for 30 min. in water bath. The absorbance of supernatant was checked at 560 nm on colorimeter. Saline was treated as blank. Aspirin (1mg/ml) was used as standard for the reaction. The experiment was done in triplicate IC50 values were calculated for formulation study. Percent inhibition of haemolysis was calculated using following formula,

$$\text{Percent inhibition of Haemolysis} = (A_{Blank} - A_{Test}) / A_{Blank} * 100$$

Where A_{Blank} = Absorbance of blank
A_{Test} = Absorbance of Test

2.7.2. Estimation of Protease Inhibition Activity by Using Azo-casein as Substrate

Proteinase enzyme released during lysis process breaks down the proteins, resulting in the inflammation, thus inhibition of proteinase enzyme is important. The anti-inflammatory agent plays the role of the inhibition of the proteinase enzyme and reduce the inflammation. The assay is based on the digestion of the Casein by trypsin. Digestion of the casein is dependent on the amount of trypsin present in presence of inhibitor. 200 µl of 0.06 mg/ml trypsin in 20 mM Tris buffer was added in test tubes. 200 µl of test extract was added in the trypsin solution. Tris buffer was used instead of test extract in blank. Incubation was done at 37°C for 5 min. Then 200 µl of Azocasein (0.08 mg/1.5 ml) was added in the reaction mixture. The tubes were incubated at 37°C for 20 min. 400 µl of 5% TCA was added in the mixture to stop reaction. Centrifugation was done at 13500 rpms for 20 min followed by addition of 400 µl of 500 mM NaOH. More the inhibition less colour will be developed. IC50 value was calculated for selected potent strains. Per cent inhibition was calculated using following formula.[15]

$$\text{Percent Inhibition} = (A_{Blank} - A_{Test}) / A_{Blank} * 100$$

Where A_{Blank} = Absorbance of blank
A_{Test} = Absorbance of Test

2.8. Preparation of Water in Oil Cream Formulation

The hair nourishing cream formulation was prepared by adding water phase in oil phase. The phase A white petroleum jelly, emulsifying wax and stearyl alcohol and Phase B ingredients sodium lauryl sufate, glycerine and water are heated separately at 85°C. The formulation was prepared by adding phase B to phase A, water was added slowly with constants stirring. Phenoxyethanol with Ethylhexyl glycerine (90:10) was used as the preservative and pH adjustment was done by using citric acid to 5.5-6. The 0.1% whole cell extract of the algal isolate LS01 was suspended in DMSO (Dimethyl sulfoxide) and added to the formulation after colling the formulation at 45°C (Table 3).

The formulation was evaluated for the accelerated stability study at 25°C, 45°C and 5°C at 70% humidity. The formulations were evaluated for the organoleptic, physicochemical, and microbiological characteristics. The odour, colour, and appearance were tested under organoleptic characteristics. pH, viscosity, and phase separation were tested under physicochemical characteristics for the period of 6 months.

3. RESULTS AND DISCUSSION

3.1. Sampling and Isolation of Strains and Growth Medium

The soil and water samples were collected from Lonar lake for the isolation of the *Leptolyngbya species* (Figure 1). The sample was enriched in the bold basal media having pH 11.0 and salt concentration was 1.5%. The microscopy confirms the isolates as *Leptolyngbya spp* (Figure 1A and 1B).

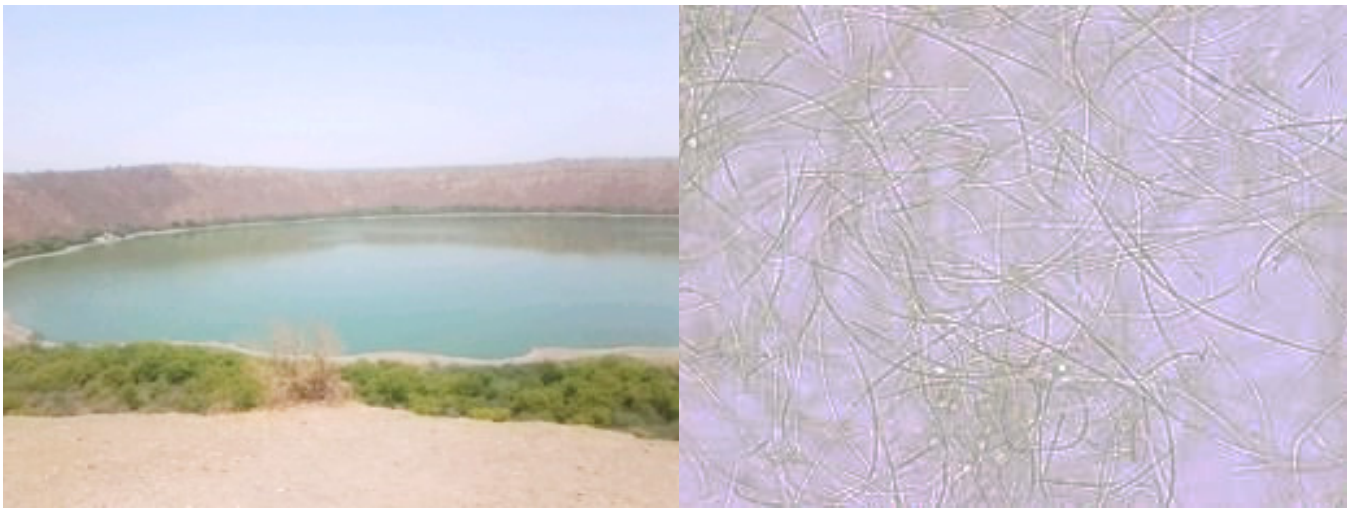

Figure 1A: Sampling site: Lonar lake

Figure 1B: LS01: *Leptolyngbya* spp.

Figure 1: Images for the sampling site and microscopic observation of the algal isolate

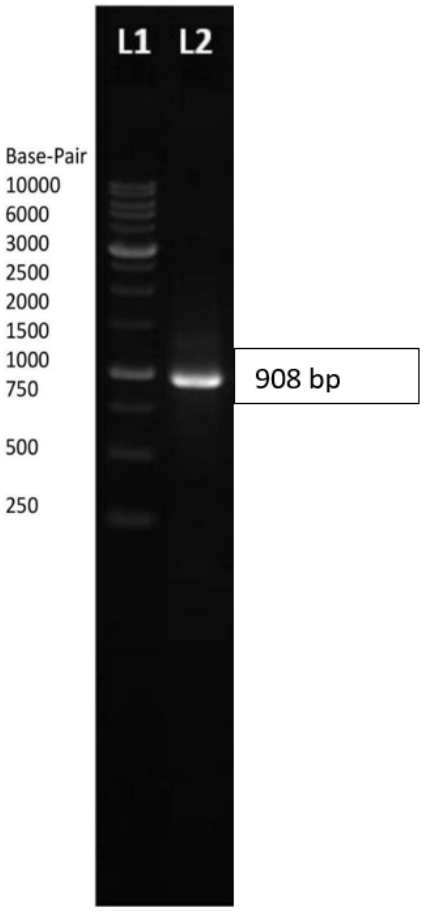

Figure 2: Electropherogram of the 16S rRNA of algal isolate

3.2. Identification of the Isolate by 16S rRNA Technique

The genomic DNA was extracted using CTAB technique and electropherogram is given in the Figure 2. Using the cyanobacterial primer. The obtained sequences were analysed using (Molecular evolutionary genetics analysis) MEGA 11 for the generation of phylogram. The sequences are given in the Figure 3. Maximum likelihood analyses were performed using MEGA 11. The program

>MW489568 *Leptolyngbya foveolarum* LS01A1 16S ribosomal RNA gene, partial sequence

ACCTAGGCGACGATCTGTACTTGGTCTGAGAGGATGACCAGGCACACTGGAACT-
GAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTCCGCAATGGGC-
GCAAGCCTGACGGAGCAATACCGCGTGAGGGAGGAAGGCTCTTGGGTTGTA-
AACCTCTTTTATCAGGGAAGAAAAGATGACGGTACCTGATGAATCAGCATCGGC-
TAACTCCGTGCCAGCAGCCGCGGTAATACGGGGGATGCAAGCGTTATCCGGAAT-
TATTGGGCGTAAAGCGTCCGTAGGTGGTTTCACCAGTCTGTTGTCAAAGCGTGC-
GGCTCAACCGCATACGGGCAATGGAAACTGTGAGACTAGAGTGCGATAGGGGT-
CACAGGAATTCCCAGTGTAGCGGTGAAATGCGTAGATATTGGGAAGAACAC-
CAGCGGCGAAAGCGTGTGACTGGGTCTGCACTGACACTGAGGGACGAAAGC-
TAGGGGAGCGAAAGGGATTAGATACCCCTGTAGTCCTAGCCGTAAACGATGACAAC-
TAGGTGTGGTTCGTATCGACCCGAGCCGTGCCGTAGCCAACGCGTTAAGTTGTC-
CGCCTGGGGAGTACGCACGCAAGTGTGAAACTCAAAGGAATTGACGGGGGCCC-
GCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTAC-
CAAGGCTTGACATCCTGCGAACTTTTCTGAAAGGAGAAGGTGCCTTCGGGAGCG-
CAGAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTA-
AGTCCCGCAACGAGCGCAACCCACGTTTTTAGTTGCCAGCATTGAGTTGGGCACTC-
TAGAGAGACTGCCGGTGACAAACCGGAGGAAGGTGTGGATGACGTCAAGTC

Figure 3: Multiple sequence alignment for isolate Leptolyngbya

Model test 5 was utilized to find the best-fit substitution model. The best sequence evolution model was Kimura 2-parameter+Gamma distribution. Pairwise distances between in groups were calculated in MEGA 11. The genome sequence was submitted to the NCBI. The isolate was identified as *Leptolyngbya foveolarum* and accession number was assigned as MW489568.

3.3. The TPC and TFC of the *Leptolyngbya spp*

The total polyphenolic content of the *Leptolyngbya spp* was estimated using the Folin-Ciocalteu method. The tannic acid was used as the standard and polyphenol content was expressed in terms of the mg/equ. of tannic acid/gm. Polyphenol content in the *Leptolyngbya spp* was found to be 27.331 ± 0.41 mg/equ. of tannic acid/gm.

The Flavanoids content of the *Leptolyngbya spp* was also estimated by using aluminium chloride method. The quercetin was used as the standard for the reaction and flavonoid content was expressed in terms of the mg/equ of Quercetin/gm. The flavonoid content of the isolate was found to be 40.076 ± 4.13 mg/ equ of Quercetin/gm (Table 1).

The polyphenol contribute in the antioxidant activity of the extracts.[16]

3.4. Antioxidant Activity of the Algal Extracts Using DPPH Assay and ABTS Assay

Antioxidant activity of the whole cell extract of the isolate LS01A1 was detected as 94.30 ± 1.83 percent inhibition for ABTS assay and 63.70 ± 0.58 percent inhibition for DPPH assay. The percent antioxidant activity for ascorbic acid (0.05mM) was found to be 98.266 ± 0.23 for DPPH assay and 96.218 ± 0.39 for ABTS assay (Table 2). The ABTS assay is more sensitive for identifying antioxidant activity because of the faster reaction kinetics and its response to antioxidants, which is higher compared to DPPH assay.[17] The antioxidant molecule neutralises the effect of the harmful free radical in the cell, thus allowing the cells to grow.

3.5. Anti-inflammatory Activity of the Whole Cell Extract of the Algal Isolate

The anti-inflammatory activity of the algal isolates was studied by using HRBC membrane stabilisation method. The prevention of hypotonicity and heat induced HRBC membrane lysis was taken as factor to evaluate the anti-inflammatory activity.

3.5.1. Hypotonicity Induced Haemolysis

The membrane stabilisation of Human Red Blood Cells for hypotonic solution induced haemolysis was estimated in presence of the whole cell extract of the algal isolate. Aspirin (1 mg/ml) was used as standard drug and it showed 98% membrane stabilisation for the hypotonic solution induced haemolysis (Table 3). The whole cell extract of the algal isolate LS01 showed 86% stabilisation with the standard deviation of 0.7%.

3.5.2. Heat induced haemolysis

Membrane stabilisation percent is directly proportional to the anti-inflammatory activity. Higher the stabilisation more is the anti-inflammatory activity. The Aspirin (1 mg/ml) was used as the standard and it showed 98% membrane stabilisation.

Whole cell extract of the algal isolate LS01 showed higher percent stabilisation for heat induced HRBC stabilisation assay. LS01 showed stabilisation as 96% ± 0.1 S.D. The experiment was done in triplicate (Table 3).

3.5.3. Trypsin inhibition assay

Trypsin and chymotrypsin are the serine proteinase digestive enzymes and are responsible for the providing nutrient to microorganism through digestion of proteins in to peptides. The anti-inflammatory activity of the aspirin was estimated at the conc. of 1mg/ml as standard drug for experiment. Aspirin showed 89 % inhibition for 250 µg/ml concentration. The whole cell extract of the algal isolate LS01 showed 50 % inhibition with 1.8% standard deviation (Table 3).

From the above data it has been observed that the whole cell extract of the alga LS01 is having higher anti-inflammatory and also possess antioxidant activity. The IC50 value calculated for the whole cell extract of the alga LS01. Whole cell extract of the algae showed 453 ± 3 µg/ml IC50 for the heat induced HRBC membrane stabilisation, 86 ± 5 µg/ml IC50 for the hypotonicity induced HRBC membrane stabilisation and 998 ± 7 µg/ml IC50 for the trypsin inhibition assay (Table 4).

3.6. Stability of the Hair Nourishment Cream

The hair nourishment cream was formulated using 0.1% whole cell extract of algal isolate LS01. The hair nourishing cream prepared with the algal extract of the LS01 isolate showed no variation in the colour, odour and appearance throughout the stability period of 6

Table 1: Data for the TPC and TFC of the *Leptolyngbya spp.*

Isolate ID	*Polyphenols mg/equ. of tannic acid/gm*	*Flavonoids Mg/equ of Quercetin/gm*
Leptolyngbya foveolarum	27.331 ± 0.41	40.076 ± 4.13

TPC, Polyphenols; TFC, Flavonoids.

Table 2: Data for the antioxidant activity of the *Leptolyngbya spp*

	Percent inhibition %	
Isolate ID	*DPPH assay*	*ABTS assay*
Leptolyngbya foveolarum	63.70 ± 0.58	94.30 ± 1.83
Ascorbic acid	98.266 ± 0.233	96.39 ± 0.39

Table 3: Data for the anti-inflammatory activity of the algal isolates

	Percent stabilisation %		
Algal isolate	*Trypsin assay*	*Heat induced HRBC stabilisation assay*	*Hypotonicity induced HRBC stabilisation assay*
LS01	50 ± 1.8	96 ± 0.1	86 ± 0.7
Aspirin (Std)	89 ± 0.41	98.49 ± 0.18	98.49 ± 0.14
	(n=3) (Percent stabilisation ± Standard deviation)		

HRBC: Human red blood cells

Table 4: Data for the IC50 value for the algal extract of LS01 for 3 different methods of anti-inflammatory activity.

Sr. No.	Evaluation method	Equation	R^2	IC50 value (µg/ml)
1	Heat induced HRBC membrane stabilisation	y = 0.0925x + 7.805	0.98	453 ± 3
2	Hypotonicity induced HRBC membrane stabilisation	y = 0.0431x + 46.093	0.97	86 ± 5
3	Trypsin inhibition assay	y = 0.0433x + 4.7688	0.99	998 ± 7

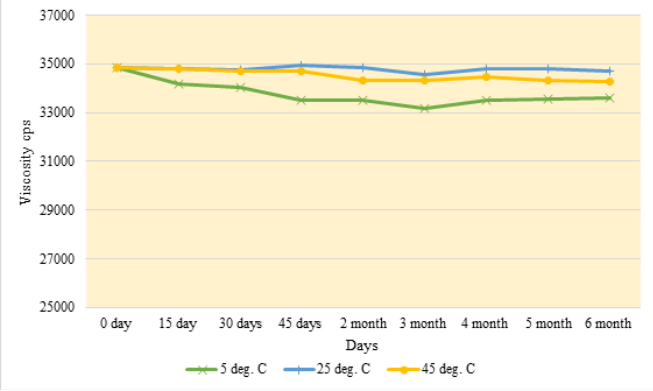

Figure 4: Graphical representation for the viscosity of the hair cream prepared with the whole cell extract of alga LS01

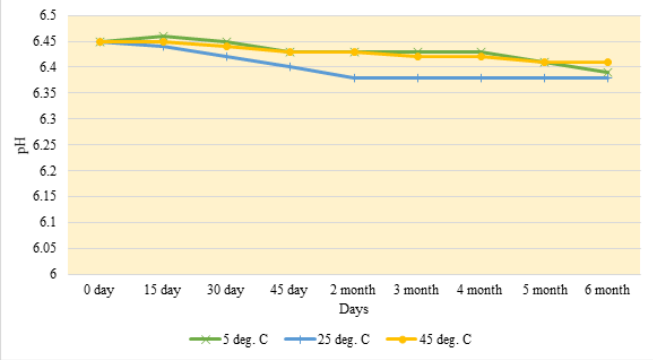

Figure 5: Graphical representation for the pH of the hair cream prepared with the whole cell extract of alga LS01

month. No sign of phase separation was observed in the formulation for 6 months stability period (Table 3).

3.6.1. *Physicochemical Characteristics*

The hair nourishment cream prepared with the algal extract of the LS01showed no variation in the pH of the formulation. The viscosity of the formulation was 37829 cps at 0 day changed to the 34284 cps at 6 months at 45°C indicate the formulation is stable for 6 months. No change was observed in the phase separation and flocculation in the formulation throughout the stability study (Figure 4 and 5).

3.6.2. *Microbiological characteristics*

The formulation was also tested for the microbiological total aerobic count for the period of 6 months no contamination was observed in the formulation during the study period.

4. DISCUSSION AND CONCLUSION

Polyphenol and flavonoids are frequently found in various plant, algae, and seaweeds. The algae serves as rich source of the phenols, flavonoids, and alkaloids which mainly contributesto the antioxidant activity.

In the present study, results interpret that *Leptolyngbya* species isolated from the extreme ecological habitat possess anti-inflammatory and antioxidant properties. These anti-inflammatory and antioxidant activities may be contributed due to the presence of phenolic, flavonoids and other metabolites. The extracts act as free radical scavengers, inhibits the heat induced damage of HRBC membranes and hypotonicity membrane stabilisation and proteinase activity. There is scope to further purify the active biomolecules and develop it as anti-inflammatory drug which can be used for the treatment of various diseases such as cancer, neurological disorders, folliculitis, and inflammation. The suitability of the extracts was checked with the hair nourishing cream base and found suitable and stable over a period of 6 months. These *Leptolyngbya* species also contains proteins, carbohydrates, and lipids in their cells which have nutritional value which can develop in the application of nutraceuticals and cosmeceuticals.

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