https://doi.org/10.48047/AFJBS.6.13.2024. .3749-3766

African Journal of Biological Sciences

EMULSIFYING SKIN REJUVENATING AND MOISTURISING ABILITY OF EXO-POLYSACCHARIDE EXTRACT FROM HYPER SALINE ALGAE *PSEUDANABAENA LIMNETICA*.

Ashwini Tribhuvan, Manjushri A. Deodhar and Ajit.Kengar*

Kelkar Education Trust's V. G. Vaze College of Arts, Science and Commerce(Autonomous), Mithagar Road, Mulund (E), Mumbai- 400081, India.

***Corresponding Author:** ajitkengar@vazecollege.net

Article History
Volume 6, Issue 13, 2024
Received: 18June 2024
Accepted: 02July 2024
doi:*10.48047/AFJBS.6.13.2024.* .3749-3766

ABSTRACT:

Microalgae when subjected to stressful environmental conditions they release gel- like exudates known as exo-Polysacchrides. These have been able to find several applications in the fields of cosmetics for skin-rejuvenation ability and for incorporation into the skin care cosmetic formulations. The aim of this study was to study the proliferative and wound healing ability of the exo-polysaccharide extract using the MTT proliferative assay along with the in-vitro scratch assay on the swiss embryonic 3T3 fibroblast cell line. This extract was added in 5 different concentrations from 50-500 ug/ml. The vehicle control used was Citrate buffer and the positive control was Standard PBSA. Physico-chemical properties exhibited by the microalgae of forming hydrogels which could be used in the cosmetic formulations as mositursing agents, stabilising agents, viscosity enhancing agents and emulsification agent. The syntetic emulsifier in the O/W emulsion was replaced by in the present research. The exo-polysaccharide extract was also tested for its moisturizing efficacy studies using the scalar moisture checker instrument. From the proliferation assay results, it can be concluded that the extract on the addition into the 96-well plate was found to stimulate the proliferation and migration of the cells at higher concentrations up to 400ug/ml of the extract without any cytotoxicity to the cells after 24 hours of exposure with the added exo-polysaccharide extract of *P.limnetica*.

From the findings it can be concluded that the cyanobacterial *P.limnetica* polysaccharide extract will be beneficial to treat various chronic wounds and other skin related wounds and it can be also utilised in the skin-rejuvenating cosmetic formulations for anti-aging. The results noted from the moisturizing ability studies prove that after 6 hours of the exposure time the moisturizing ability increased to 24.64±0.779 as compared to control reading of 22.56±0.669. The increase was found to be nearly to 6- 8% on the addition of the 0.1% of the *Pseudanabaena limnetica* exo-polysaccharide extract in the cosmetic formulations.

KEYWORDS

Proliferation, fibroblast cell line, anti-aging, cosmetic formulation, exo-polysaccharide extract, *Pseudanabaena limnetica*, skin rejuvenating ability.

1. INTRODUCTION

The carbon dioxide emission into the atmosphere from the industries along with the bioremediation of water from the sewage tanks can be curbed with the help of algal production. Fast growing microalgae production as an alternative and biodiesel and bioethanol production from the lipids and carbohydrates. But there are many practical difficulties in making the project cost effective. **(Lam and Lee, 2011).** Polysacchrides , fatty acids, proteins, carotenoids are the several pigments obtained from algal source . Biorefeinary concept has been a boon to the algal industry . **(Bhowmick and Sen, 2018).**

For the cost effective production of *P.limnetica* strain it was cultivated into the 1000L capacity photobioreactor. Under the natural conditions of Mumbai region this halophilic *P.limnetica* strain was cultivated in the higher tempeartures of 45°C and the light intensity of (65,000-85,000 lux), **(Magar and Deodhar ,2018**).The non essential CO_2 was scrubbed out the system in the BIO-CNG production. (**Rambhiya et al, 2021)**

Under high light intensity and exposure to UV radiations, exudates of high amount of extracellular polysaccharides (EPS) is one of the common protective mechanism in algae. Carragenen , Agar-agar, glucans and ulvans several distinct properties are possessed by these algal polysacchrides. Algal polysaccharides possess skin lightning , moistursing , UV protective , anti-wrinkles providing properties. Moreover, they have a wide spectrum of physico-chemical properties, such as the ability to form hydrogels, which extend their utilization as emulsifiers, stabilizers, and viscosity controlling ingredients in cosmeceuticals.

The present study deals with exo-polysaccharide extracted from *P. limnetica.* It deals with cell-regenerative ability of polysaccharides on 3T3 dermal fibroblasts. The ability of the polysaccharides derived from *P. limnetica* strain, as a bio-based emulsifier was also studied .The polysaccharide is incorporated in cosmetic cream formulation and its moisturising ability is evaluated.

2. MATERIALS AND METHODS

MICROALGAE SPECIES (*PSEUDANABAENA LIMNETICA*) SAMPLE COLLECTION

The algae sample collection of the strain *P.limnetica* was done from the salt-pans of Mulund, Mumbai areas**.(Mhaskar K and Deodhar M, 2016**). The scaling up of the same strain was done using 60L photobioreactor system under physico-chemical parameters as described by **(Magar and Deodhar, 2018).** This strain was cultivated in the modified SW-BG11 medium for efficient growth to obtain optimum biomass and copious amounts of polysaccharides.

EXTRACTION OF EXO-POLYSACCHARIDES FROM *P.LIMNETICA*

The algal biomass was subjected to Hot water extraction technique. About 500 ml of the algal culture was centrifuged to get about 5-6 gms of wet biomass. The biomass was in the ratio 1:1 was resuspended in the water and then heated at 85°C-45 minutes in beaker. Biomass Centrifugation of the algal biomass was done at 6000RPM for 15-20 minutes for obtaining the supernatant for the extraction of the exo-polysacchrides .

EFFECT OF EXOPOLYSACCHARIDES EXTRACTED FROM P. LIMNETICA ON TOXICITY/CELL PROLIFERATION OF DERMAL FIBROBLASTS IN VITRO BY MTT ASSAY

3T3 Fibroblast Cell Culture:

From NCCS Pune the 3T3 fibroblast cell line were procured. DMEM medium was utilised to maintain these fibroblast cell lines along with 10% FBS.

MTT Assay For Cell Viability /Cytotoxicity:

96 well plate was used for the seeding of the fibroblast cells containing the concentration of $1x10^3$ cells/ml. The concentration ranging from 50-500ug/ml was added to the wells. 10ul out of each of the test concentrations was added. Each of the concentration was set in triplicates. A cell grown in DMEM supplemented with citrate buffer was used as a vehicle control. The cells grown in the PBSA were used as the positive by control. 1% SLS was used as negative control. Cells were then incubated with 5mg/mL MTT (Hi-Media) for 4h at 37°C. DMSO of 100µl was injected into each well and OD was noted using the Elisa plate reador at 570nm.

Scratch Wound Healing Assay:

Cell-cell migration was studied using the 3T3 fibroblast cell line . The fibroblast cells with the density of $1x10^6$ cells/ml were seeded into the 6-well tissue culture plate, (**Sigma-Aldrich**) to attain 70-80% confluency. 20 µl sterile tip was used for striking a linear scratch in the monolayer. PBSA was used for the debris removal if any. The temperature of 37°C was maintained inside the incubator with 5% CO_2 for 24 hours. The control scratch well was supplemented with same amount of DMEM. The cell migration was studied at the interval of 0 hour, 3 hours and 24 hours on inverted microscope provided with camera.

Microscopy And Imaging:

Inverted light microscope was used to study cell-cell migration after (0hr,3hr and 24 hours) intervals. **Image J** software **(Scion Corp., Frederick, MD, USA**) was utilised to measure the width of the scratch area after (0, 3 and 24) hours time interval.

Statistical Analysis:

Anova tool was used to study the date using statistical analysis.(a=0.05). The statistical analysis would effectively state the statistical differences between the control (Vehicle control) and the test samples**.(test concentrations**).

TO STUDY EMULSIFYING ABILITY OF P. LIMNETICA POLYSACCHARIDE IN O/W EMULSIONS

Preparation Of the Emulsion:

The *P.limnetica* polysaccharide extract was obtained using the Hot-water extraction methods described earlier. The O/W emulsion was prepared by mixing the two phases such as oil and water phase. Emulsions of the sesame oil, coconut oil and mineral oil in the ratio of 0.2ml:10 ml of water with 0.08% of natural emulsifier from the *P.limnetica* polysaccharide were prepared. The emulsion

SERIALNO:	***INGREDIENTS:***	***QUANTITY.(gm%)***
WATERPHASE:		
1.	Water	88.4
2.	Glycerin	4
3.	Methyl Paraben	0.2
4.	E.D.T.A	0.2
OILPHASE:		

was stirred with magnetic stirrer for 10-15 minutes. The stability of the emulsion was studied after 7 days.

Replacing The Synthetic Emulsifier With Natural Emulsifier *P.Limnetica* In The Cream Formulation:

The ingredients used for the preparation of the cream formulations were of the analytical grade. The composition of cream formulation is given in **Table 1**. The oil phase and the water phase were blended at 75°C-80°C. Stability testing of the cream formulation was done by storing the formulation at room temperature. The different parameters observed during the storage were any signs of the physical instability such as creaming, breaking of the cream formulation , phases separation of the cream.

5.	Coconut oil	1
6.	CCTG	2
7.	Cetylalcohol	3
8.	B.H.T	0.2
Emulsifiers Used :		
Formulation 1	Stearic acid	1
Formulation 2	*P. limnetica* polysaccharide	1
Formulation 3	Stearic acid concentration Polysaccharide concentration	0.50 0.50
Formulation 4	Stearic acid concentration Polysaccharide concentration	0.334 0.166
Formulation 5	Stearic acid concentration Polysaccharide concentration	0.375 0.125

Table 1: Composition of Cream Formulation

Extraction Of Exo-Polysacchrides From *P.Limnetica*:

For the extraction of Polysacchrides same Hot-water extraction method described earlier was followed. But the time and temperature conditions were standardised to optimise the yield of polysaccharide. The extraction was carried out at 3 different temperatures such as 65°C, 85°C and 100°C at all these temperatures time parameter was varied for 30 minutes, 45 minutes and 60 minutes.

Cosmetic formulation for the cream base:

The polysaccharide extracted at 9 different time and temperature condition was incorporated in cream base mentioned earlier which consisted of 1% stearic acid and 0.1% polysaccharide.

EVALUATION OF THE MOISTURISING ABILITY USING THE SCALAR MOISTURE CHECKER

The algal polysaccharide of *P.limnetica* extracted at various time and temperatures as per the protocol described previously. Cream formulated from each extract of polysaccharide was prepared and evaluated for moisturizing efficacy further using the Scalar moisture checker **(MY-808S)**. 0.01gms of each cream was applied on 2 areas. The third area was marked as blank. The moisturizing ability was measured using the scalar moisture checker immediately after the application of the product, and after 2 hours , 4hours and 6 hours.

CHARACTERISATION OF THE *P.limnetica* EXO- POLYSACCHRIDE EXTRACT USING FT-IR ANALYSIS

The P.limnetica polysaccharide extract was subjected to the FT-IR analysis. The analysis of the exo-polysaccharide extract was analysed from 400-4000 cm^{-1} spectr

3. RESULTS AND DISCUSSION:

Effect of Exopolysaccharides Extracted from P. Limnetica On Toxicity/Cell Proliferation of Dermal Fibroblasts In Vitro By MTT Assay:

One of the most profitable industries being that of cosmetic the consumers prefer natural products over the artificial ones . Bioactives obtained from natural polysaccharides such as animals, plants have obtained major attention among consumers.

Algal polysaccharides such as ulvan, agar-agar can be used anti-aging along with sun protecting factor .Many of them are used as thickeners emulsifiers and viscosity controllers.

Skin -ageing is associated with degradation of extra-cellular matrix in both epidermal and dermal layers. Though it is governed by genetic or intrinsic factors several extrinsic factors also contribute to it such as they include exposure to UV radiations ,light, smoking Et cetera. The thickness of the skin reduces due to process of aging . Elasticity of skin decreases which leads to wrinkles on skin.

The cells such as macrophages , fibroblast , kerationcytes produce enzymes containing MMP'S. Some of the bioactive are matrix metalloproteinase inhibitors and they control the ageing process by controlling degradation of matrix metalloproteins. But there are certain biomolecules which alters activity of fibroblasts and enhance production of collagen, elastin and proteoglycans and improves the appearance of the skin. **Natural polysaccharides like Aloe vera gel, Chitosan, Hyaluronic acid are fibroblast activators .** The Effect of polysaccharides extracted from *Codium edule* on production of MMP- 1and UV -induced skin damage has been extensively studied by (**Vasquez et al,2019)**

In the present study, the cell rejuvenating activity of polysaccharides extracted from *P. limnetica* has been studied.

Cultivation of *P. limnetica:*

Isolation of P.limnetica species was carried out from Mulund saltpans around Mumbai areas. **Mhaskar K and Deodhar M, 2016**). The scaling up of the same strain was done in 60L large photobioreactor systems in SW-BG11 medium as described by(**Magar and Deodhar, 2018)**.This strain was further subjected to Hot-water extraction technique to obtain Exo-Polysacchrides.

MTT Assay For To Study Cytotoxicity/Cell Rejuvenation Ability Of Polysaccharides From P. Limnetica On 3T3 Dermal Fibroblasts:

SR. NO.	***EXTRACT TEST CONCENTRATIONS: ug/ml***	***OD READINGS(570NM)***	***PROLIFERATION. (%)***
1.	50	0.490 ± 0.0006^{e}	111.36 %
2.	100	0.513 ± 0.0011^{d}	116.54 %
3.	200	0.743 ± 0.003^{b}	168.86 %
4.	400	0.763 ± 0.0006^{a}	173.40 %
5.	500	0.63 ± 0.0006^{c}	143.18 %
6.	Citrate buffer-Vehicle	0.44±0.0006	100 %

	control		
7.	PBSA	0.55±0.0006	125 %
8.	1%SDS	0.140±0.009	-

Table 2: ***Effect Of Polysaccharide Extracted From (P.Limnetica) On 3t3 Fibroblast Cell proliferation***

The assessment of the metabolic activity was done using the MTT assay . Viabilty of the cells is determined by the NAD(P)H enzyme .(**Stocket et al, 2018).** Purple colour presents the presence of formazon cyrstals wich is converted from yellow tetrazolium dye MTT enzyme . Enahcement in purple colour indicates increases presence of formazon cyrstals .Effect of various concentration of polysaccharides was studied extracted from P.limnetica .$1x10^3$ cells were seeded into the 96 well plate.

50-500ug/ml concentration of the P.limnetica polysaccharide extract was injected in the well and stored for 24 hours. On day 3, the MTT reagent was added in the concentration of 10 ul and the 96-well plate was stored in dark for 3-4 hours and later the viability of cells were checked at 570 nm using an Elisa plate reader. The table no 2 shows the effect of various concentrations of the polysaccharide extract on the fibroblast cell line. Cells grown in DMEM supplemented with citrate buffer was used as a vehicle control. The cells grown in the PBSA were used as the positive by control. SLS was used as negative control.

The viability of 3T3 fibroblasts cultures in DMEM and citrate buffer was treated as control.(100%) The viability of the cells PBSA as positive control was found to be 125%. On addition of 50 ug/ml of the polysaccharide extrsct the proliferation obtained was 111.36% and OD was found to be 0.490 ± 0.0006 (significantly increased by p>0.05).

As the concentration of the extract increased up to 100 ug/ ml the optical density was 0.513±0.00115 which did show a percent proliferation rate of 116.59%.(proliferation increased significantly by p>0.05).The maximum viability of the cells was at the concentration of 200 ug/ml concentration of 0.743 ± 0.003and % proliferation was as high as 168.86%.(Proliferation significantly increased by p>0.05)

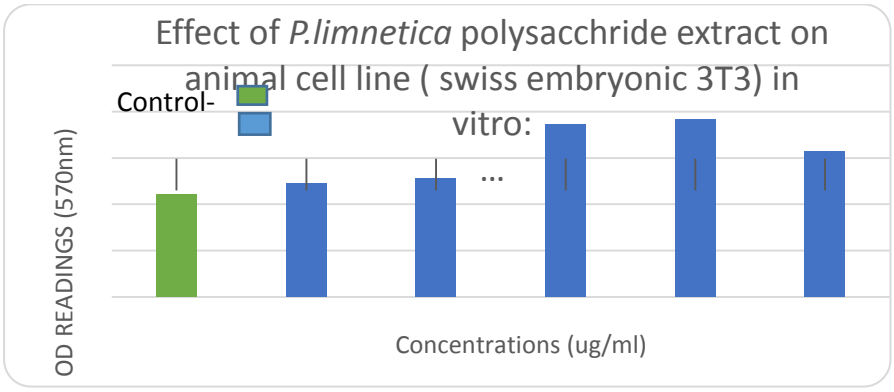
On further addition of the extract at 400 ug /ml concentration the cell viability found was 0.763±0.0006 and the rate of proliferation was 173.40%. Significantly increased by p>0.05) On addition of 500 ug/ ml of the extract there was a little decline in the viability was found to be 0.63±0.0006 and % proliferation rate was 143.18%. Up to the 500ug/ml the percentage proliferation decreased significantly at p>0.05. But it was higher than both DMEM control and the PBSA control. Thus, the concentrations up to 500 ug/ml were not at all cytotoxic but proliferative in nature.

From the results above it can be inferred that the MTT experiment results were shown in the form of mean ± SD format. The data set contains the average readings of the experiments carried out using the triplicate readings. From the parametric tests that was applied to this experiment of one-way ANOVA analysis , which states the significant statistical difference between the controls and

the test concentrations (à= 0.05)**Fig no: 1.** It can be inferred that the data is significant at the(à=0.05 level of significance)

Figure 1: ***Effect Of Polysaccharide Extracted From (P.Limnetica) On 3t3 Fibroblast Cell Proliferation:***

(*signifies the difference found by the one-way Anova analysis , (p<0.05)



Effect Of Exopolysaccharides Extracted From P.Limnetica On Cell Migration And Wound Healing In Vitro:

Wound healing assay was performed to study the fibroblast cell migration using the **Image J** assay software after an interval of 3hrs and 24 hrs. (**Gabbiani et al, 1984).**

SR.NO:	***CONCENTRATIONOF EXTRACT(ug/ml)***	***RATE OF WOUND HEALING(%).***
1.	Control 3 hours	4%
2.	Control 24 hours	20%
3.	100 Test 3 Hours.	69%
3.	*100 Test 24 Hours.*	77%
4.	*200 Test 3 Hours.*	74%
5.	200 Test 24 Hours.	82%

Table No. 3: ***Percent Wound Healing Activity Of The Exo-Polysacchrides Extracted From P.Limnetica Strain***

The results are depicted in tabulated format and the amount of cell migration in control and treated cells in % is tabulated in Table 3 above. As seen in table, the control cells showed negligible growth at 3 hours, it was 4% and after 24 hours it was only 20% respectively. Fibroblast cells supplemented with 100ug/ml of exopolysaccharides showed enhanced growth compared to control. It was 69% after 3hrs and after 24 hours it was found to be 77%

In the cells supplemented with 200 ug/ml of exopolysaccharides, cell migration was still higher after 3 hours which was 74% and after 24 hours it was 82%.

Results confirm the ability of exo -polysaccharides for cell proliferation and migration.

Polysaccharides are involved in several pharmacological activities such as cell adhesion, inflammation, angiogenesis and anticoagulation. They contribute in immunomodulatory activities through modulating functions of macrophages. This include activation of phogocytosis, increasing cytotoxicity against tumor cells and reactive oxygen species. cytokines such as IL-1, IL-6 and TNF alpha are secreated from the polysaccharides .**(Li et al, 2018).**

VEGF, FGF aide in the fibroblast proliferation and migration and TGF regulated collagen synthesis.These growth factors play a role in wound healing **.(Li et al ,2018).**

Effect of fucoidans, the sulphated polysaccharides extracted from the brown algae on the proliferation of fibroblast and reconstruction of the skin equivalents was studied by **(Sang et al,2014).**Viabilty of bthe cells were checked by MTT assay for 3 days with 0-50mg/ml of fucoidan along with the CCD-25 human fibroblast cells. When the cells were treated with 1 mg/ml of Fucoidans, the cell proliferation increased by 20%. At the 10 mg/ml there was a little decline in viability of the cells. But at the concentration 50 mg/ml of Fucoidans there was a significant decline in the viability of the cells. Only 60% cells were viable. Also, at the 10 mg concentration, the fucoidans increased the expression of the cyclin dependent Kinases cyclin D, which regulates the cell cycle. A major contributor to aged skin are the fucoidans exhibiting increase in the expression of MMP-1 and type 1 pro-collagen.(**Moon et al, 2009).**Thus Fucoidans do not play much role in rejuvenating fibroblasts or adding the matrix metalloproteins. But it controls the production of matrix metalloproteinases and saves MMP's like collagen or elastin from degradation thus contributing to anti-aging.

Xyloglucans are the polysaccharides extracted from the tamarind seeds. They have cellulose, B-1,4glycoside backbone with side chains containing arabinose, rhamnose, xylose residues.

(**Nie and Deter, 2013)** extensively studied the ability of tamarind Xyloglucans in fibroblast proliferation promotion of the cell cycle and stimulated migration of the cells which leads to skin regeneration. NHDF , NHEK AND HaCAT were used for the dermal proliferation assay. On all the three cell lines concentrations of xyloglucans ranging from 0.01ug/ml to 100ug/ml were used. All concentrations contributed to significant proliferation over the control. A scratch was done on the monolayer on the HDF containing Tamrind xyloglucan to study the effect of xyloglucan.

Our polysaccharides extracted from *P. limnetica* can also be considered as a natural source contributing in rejuvenation of fibroblasts .

Emulsification Ability Of The Exo-Polysacchrides Extracted From The Strain P.Limnetica :

Emulsions are such in which one phase is dispersed into the other along with portraying of therapeutic properties which was subdivided into two types O/W emulsions and W/O emulsions .For treating the dry skin emolillents containing W/O emulsions are used. **(Smaoui et al, 2012).**

Emulsions are thermodynamically unstable and tend to break down during storage . It is indicated by a variety of physicochemical changes like creaming, flocculation and coalescence. The stability of the emulsion can be extended by addition of various stabilizer , emulsifiers or thickening agents which include Tween 80, Lecithin, xanthan gum , Guar gum, Gum Arabic, lactoglobulins, c-carrageenan , glycerine fatty acids Et cetera. **(Semenzuto et al, 2018)** . Due to several added benefits such as maintenance of human health , environmental friendly , reduction in animal cruelty there is an inclination of consumers towards plant based emulsifiers and also show various other such properties such as non-inflammation , non bacterial properties.

The stability of the O/W emulsions or W/O emulsions can be deciphered with the various destabilizing processes such as coalescence , flocculation , creaming or phase inversion that the emulsions undergo after different time rates. In the current study the three types of O/W (0.2ml:10ml) emulsions of mineral oil, sesame oil and coconut oil were prepared. 0.8% of the P.limnetica polysaccharide extract was then incorporated into three different emulsions mentioned above. These 3 different O/W emulsions were further stored for 7 days for a stability check studies with respect to change in droplet size , phase separation and flocculation. Out of the three different oils used , the emulsion prepared using coconut oil did show a uniform droplet size from day 1 to day 7. In the coconut oil emulsion, the appearance of the emulsion was clear and there was no phase separation observed or flocculation seen even on day 7.

SerialNo.	**Concentration of Stearic Acid (G/100Gms of TheFormulation)**	**Concentration of the *P.limnetica*Extract. G/100Gms Of The Formulation**	**Status of The Cream Formulation:**
1.	1	-	Good Consistency
2.	-	1	Watery consistency
3.	0.5	0.5	Good consistency
4.	0.334	0.166	Lesser consistency
5.	0.375	0.125	Lesser consistency

Table 4: Variation in the concentration of the natural emulsifier extract (P.limnetica) and synthetic emulsifier, (Stearicacid) in the cream formulation.

The emulsification ability of the *P.limnetica* extract was studied by replacing the synthetic emulsifier (Stearic acid) with the natural emulsifier in the 4 different cosmetic formulations. Hence we decided to prepare a coconut oil based cream formulation. And while preparation of the same our objective was to replace the artificial synthetic emulsifier Stearic acid with the natural emulsifier extracted from *P.limnetica* strain.

The 1st formulation prepared gave a good consistency in the presence of only stearic acid . The second formulation prepared did show an unstable consistency consisting of only the *P.limnetica*

extract. The third formulation prepared did show a good and stable consistency which consisted of 50% of the natural emulsifier of *P.limnetica* and 50% of the synthetic emulsifier, Stearic acid. The 4th and the 5th formulation prepared did show a lesser consistency as compared to other formulations.
From the above results, it can be inferred that the microalgae polysaccharide extract from *P.limnetica* that was incorporated in the cream formulations gave the better results in the formulation containing 50% of the natural emulsifier.

Number of moisturizers available under the label of natural organic or herbal use some synthetic emulsifier, humectants or occlusive agent. There is extensive need to replace these toxic synthetic agents with natural ones.

Stearic acid and sunflower wax were used for preparation of 9 variables . evaluation of parameters such as pH, particle size , viscosity was done**.(Maru and Lahoti,2018)**. Best results were seen in the formulation containing 2% stearic acid and 2%sunflower wax. In conclusive remarks they wrote : We are blessed by many magical ingredients. It depends on us how to explore them scientifically. Formulators must play an active role so that the consumer will get maximum benefit of our traditional heritage.

MOISTURIZING ABILITY STUDIES OF EXO- POLYSACCHARIDES EXTRACTED FROM P.LIMNETICA.

Stratum corneum helps regulate water loss in the lipid matrix prevents the loss of water along with the hydrophobic compound that hold water. Skin hydration and retention of water is maintained by glucosamine glycan like polysaccharides.. They are also responsible for critical skin repair and renewal . But scorching heat, extreme cold, pollution and lack of proper care makes the skin dehydrated and strongly influences mechanical properties of skin such as softness ,flexibility elasticity. Moisturizer mimic and augment skins natural water retention mechanism.

Natural polysaccharides are important class of biomolecules that find many uses in moisturizing formulations. In addition to the moisturizing effect they are highly compatible, biodegradable (**Brode, 1991).** These are available in abundance and are in-expensive. These properties of hydrocolloids help them to obtain worldwide acceptance amongst the consumers with respect to their safety and efficacy. **(Kanlayavattanakul and Lourith in 2015)** made an elaborate review on bio- polysaccharides in skin hydrating cosmetics. The review includes polysaccharides from different sources such as, microbial plant and animal derived polysaccharides and the seaweeds which are frequently used in moisturizing cosmetic formulations.

They elaborately explained the mechanism of hydration or moisturising effect of moisturiser. Moisturisers serve to return water content preventing trans epidermal water loss(TEWL). The formulated moisturizer is expected to suppress TEWL. Humectants and occulants provide combined effects when present in skin hydrating cosmetics formulations. Occlusive elements are generally emollients or lubricants which inhibit TEWL and give smoothness to the skin. With humectants skin tissue is re-moistened from inside out. Sensoral evaluation of skin moisture efficacy the practical analysis is provided by instrumentation (**Kanlayavattanakul and Lourith**

,2015 loc cit). The non-invasive methods that are accepted to be standard methods for clinical evaluation of skin hydration are: **Corneometer/Moisture-meter,** measures the skin hydration on the basis of electric capacitance or conductance. The current study deals with checking the moisturizing ability of the *P.limnetica* polysaccharide extract on human volunteers using the scalar moisture checker instrument

EFFECT OF VARIOUS TIME-TEMPERATURE COMBINATIONS ON THE MOISTURISING EFFICACY OF EXO-POLYSACCHRIDES FROM *P.LIMNETICA*

TIME - TEMPERATURE COMBINATION.	***BLANK***	***AFTER APPLICATION***	***2 HOURS***	***4HOURS***	***6 HOURS***	***%TOTAL POLYSACCHRIDE: (Gms)***
65°C-30minutes	22.88±3.120	24.76±2.439	23.08±1.164	22.54±1.128	22.2±1.372	0.06
65°C-45 minutes	20.32±3.131	22.72±1.375	22.48±1.177	21.82±0.933	20.7±1.095	0.132
65°C-60minutes	20.32±3.131	22.3±1.417	23.8±1.949	23.1±1.772	23.64±1.683	0.079
85°C-30minutes	21.96±1.188	22.3±0.827	23.58±1.416	22.6±0.624	22.46±0.870	0.088
85°C-45minutes	21.96±1.88	22.82±0.973	23.9±1.064	23.42±0.432	23.32±0.798	0.079
85°C–60 minutes	22.5±1.326	23.56±1.457	22.08±0.481	23.58±0.496	23.08±0.804	0.070
100°C-30minutes	21.24	22.4±0.902	22.54±0.965	22.2±0.827	22.98±0.739	0.112
100°C-45minutes	21.78±1.100	22.12±1.177	22.04±0.680	23.38±0.630	22.34±0.466	0.380
100°C-60minutes	22.56±0.669	23.58±1.233	23.36±0.497	23.46±0.577	24.64±0.779	0.407

Table 5: ***Effect Of The Time-Temperature Combinations On The Moisturizing Efficacy Of Polysaccharides****.*

The algal polysaccharides were extracted with the hot-water extraction method as per mentioned in 3.8.1. Extraction was carried out at three different time-temperature combinations. The temperatures that were varied were 65°C, 85°C and 100°C. The duration of the extraction was also varied from 30 minutes, 45 minutes and 60 minutes. The amount of polysaccharide extracted was

noted in **Table 6**. The moisturizing ability of the exo-polysaccharide in the cream formulations was noted. The results are incorporated in **Table no 6**.The test was conducted on 5 different volunteers. Three areas were marked on the forehand of the volunteers. 0.01gms of the cream product was applied on 2 areas. The third area was marked as blank. The moisturizing ability was measured using the scalar moisture checker immediately after the application of the product, and after 2 hours , 4hours and 6 hours. The results obtained were noted in the table no 6,

It was observed from the **Table no: 6** , that at all the temperatures 65°C, 85°C and 100°C when the duration was 30 minutes the moisturizing effect was seen immediately after application. But it lasted for only 2 hours. For example, when the extraction was carried out at 60°C for 30 minutes the blank reading was 22.88±3.220 After the application of the product the moisturizing efficacy was seen to be 24.76±2.439. After 2 hours it was seen to be 23.08±1.164 but at 4 hours and 6 hours it decreased to 22.54±1.228 and 22.2±1.372 which was less than blank reading .Similar was the case at 85°C ,when the duration of the extraction was 30 minutes. Initially the moisturizing efficacy was 21.96±1.188. After application it was 22.3±0.827 after 2 hours and increased to 23.58±1.416 But after 4 and 6 hours it decreased to 22.6±0.624 and 22.46±0.870.

Similar observations were observed when polysaccharide was extracted at 100°C for 30 minutes duration. At all temperatures when the extraction was carried out for a longer duration of 60 minutes the moisturizing efficacy remained for 6 hours. For instance, when the extraction was done at 85°C for 60 minutes the blank reading was 22.5±1.326on application it was 23.56±1.457 and after 6 hours it was 23.08 ± 0.804. Hence irrespective of the temperature, duration of the extraction of polysaccharide is important. The best results were obtained in the combination of 100°C-60 minutes. In this experiment the blank reading that was obtained was 22.56±0.669 which then increased to 23.58 ± 1.223 after application which then sustained in the same range even after 6 hours after the application of the product. At the 6^{th} hour it was found to be 24.64±0.779. There was an increase in the reading over the control by 8.78%. (5^{th} table). The table 5 also states that as duration increses more amount of polysaccharide is extracted. Thus, extraction for longer time helps in duel way. (**Choi et al,2013)** screened 12 Korean species of seaweeds for their moisturizing ability .The moisturizing effect was studied using the corneometer, the device which measures the water content of the superficial dermal layers. Firstly, to check the moisturizing ability of the seaweed extracts, the cream containing 5% extracts of each of the seaweed was applied to the forearms of 10 female individuals . Two hours after the application of the product the hydration of the skin was compared to the untreated skin. Out of the 12 screened species for the moisturizing ability 4 species showed no skin moisturizing effect compared to placebo. The 3 species showed slightly moisturizing effect (18.16 – 19.09%). The test cream containing 5% *Gracilaria verrucosa, Laminaria japonica, Porphyra vejonis , S. sagamianum and Ulva pertusa* showed considerable moisturizing activity. The increase in the percentage of hydration over the untreated skin was found to be 28.71%, 23.46%, 24.27% and 19.99% respectively. Amongst them the strongest activity was exhibited by *L.japonica.*

To identify the concentration showing the optimum activity, the various concentrations of *L. japonica* extracts ranging from 1%-15% were used. In the test cream without the extract the moisturization level was found to be 17.02%. For 1% of the extract the moisturizing ability was 21.31%. For 3% extract, the increase in the moisturizing ability was 24%. The highest increase of

10% extract was found was 31.46%. Thereafter at 15% concentration the moisturizing ability dropped to 30.72%. The moisturizing effect of the extract lasted for 8 hours. At the 8^{th} hour the moisturizing effect was found to be 18.70% whereas for the control it was 15.81%.

Our cream formulations containing the 0.1% of the polysaccharide extracted from *P.limnetica* also shows about 6-8% increase in the activity over the control and the activity lasted for almost 6 hours. The effect of the various concentrations of the *P.limnetica* moisturizing ability need to be carried out which is under process.

INTERPRETATION OF THE FT-IR RESULTS OF THE P.LIMNETICA POLYSACCHRIDE EXTRACT

The exo-polysaccharide extract was subjected to the FT-IR analysis using the (Shimadzu instrument) from the spectrum 400-4000cm^{-1}. It was observed that there was a presence of glycosamines containing the oligosaccharide. The peaks did also show an amide containing functional group along with OH group.

4. CONCLUSION

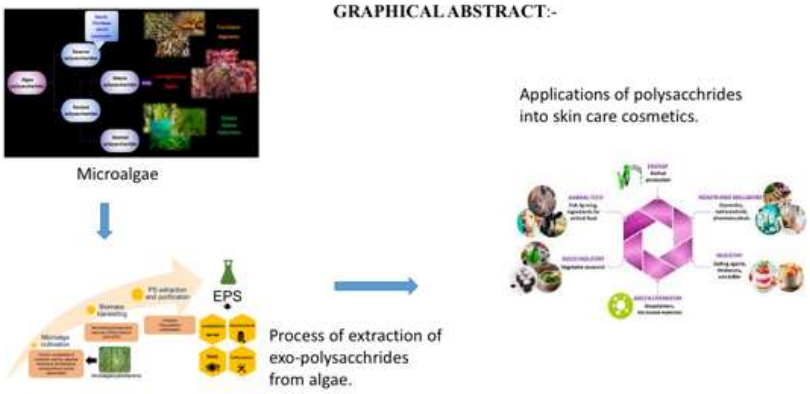
The cell viability assay was carried out for checking the cytotoxicity of the *P.limnetica* polysaccharide extract was carried out using 96- well plate from the results obtained it can be inferred that as the concentration of the polysaccharide extract in the well increases there is increase in the rate of proliferation of the cells was found up to 400 ug/ml concentration of the extract . The extract was proliferative in nature without any cytotoxicity at the higher concentrations as well. The similar findings were seen in the scratch assay which proved that with the increase in the concentration of the *P.limnetica* exo-polysaccharide extract the cell migration rate along with the wound healing percentage enhanced . Even as the exposure time increased the rate of wound healing was found to increase synergistically with the proliferation of the fibroblast cells. The other properties of the exo-Polysacchrides such as the emulsification ability and the moisturizing efficacy of the extract was tested by incorporating it into the cosmetic formulations which gave a good consistency of the even after 7 days of the stability testing in terms of the paraleptic properties as well as the results obtained for the moisturizing efficacy using the scalar checker instrument gave results at the higher time and temperature combination of 100°C-60 minutes which was 8.78% over the control sample. These properties of the extract would be beneficial for future prospects to be used in the cosmetic formulation promoting anti-aging and moisturizing properties. A more comprehensive study on the active ingredients of the *P.limnetica* extract will broaden the scope of its application in field of cosmetics and healthcare in future perspectives.

ACKNOWLEDGEMENT:
The authors also acknowledge and are thankful to the Principal, Dr.B.B.Sharma ,V.G.Vaze college of Arts ,Science and Commerce, (Autonomous), Mulund east, Mumbai for extending their support towards this work as part of my research.

DECLERATIONS:
Funding:
No funds were provided for this research work to be conducted.
Conflict of Interest:
The authors declare that they have no competing interest.
ABBREVIATION:

SR.NO	**ABBREVIATION**	**NAME:**
1.	µg/ml	Microgram /millilitre
2.	O/W	Oil in water
3.	W/O	Water in oil
4.	P.limnetica	Psedanabaena limnetica
5.	L	Litre
6.	°C	Degree Celsius
7.	RPM	Revolutions per minute
8.	Hr	Hours
9.	SLS	Sodium lauryl sulphate
10.	Gm%	Gram percent
11.	FCS	Fetal calf serum

GRAPHICAL ABSTRACT:-



The microalgal polysaccharides have been extracted using the Hot-Water extraction technique. The physic-chemical parameters were standardized to obtain the maximum production of the biomass as well as the polysaccharide content. The exo-polysacchrides properties such as Emulsifying property, Moisturising ability and Wound healing and cell proliferation activity was also checked on fibroblast cell lines in vitro. The exo-polysacchrides obtained from the cyanophycean strain P.limnetica could be used further in the commercial market in the field of skin care cosmetic applications

Ethics approval:
N.A.
Consent to participate:
All the authors have contributed equally and voluntarily in the conduct of the study and preparation of the manuscript.
Consent of Publication:
This article is sent for publication upon the consent of all the authors.
Availability of data and material:
Data generated during the conduct of this study has been included in the manuscript.

REFERENCES:

1. Lam. MK and lee. KT, Microalgae biofuels: A critical review of issues, problems and the way forward. Biotechnol Adv, 2011, May – June;30(3):673-90.
2. Bhowmick. G and Sen.R, Zero waste algal bio refinery for bioenergy and biochar: A green leap towards achieving energy and environmental sustainability, Science of the total environment, 2018, Volume 650-Part;2:2467-82.
3. Magar CS, Deodhar MA, Operational strategies for cost effective mass cultivation of halophilic microalgal strain *Pseudanabaena limnetica* in 1000-L flat panel photobioreactor, J Petrol Environ Biotechnol ,2018, 9:380.

4. Rambhiya SJ, Magar CS, Deodhar MA, Using seawater-based Na2CO3 medium for scrubbing the CO2 released from Bio-CNG plant for enhanced biomass production of *Pseudanabaena limnetica*, SN Appl Sci, 2021, 3: Article number: 276.
5. Mhaskar, K. and M. Deodhar, Isolation of halophilic cyanobacteria from saltpans of eastern suburbs of Mumbai. Int. J. Sci. Res. Methodol, 2016, 4: 37-48.
6. Ross Dizon Vasquez, De Asis Fernandes, Jovencio Garcia Apostol, Stephen Lirio, Cytotoxicity and protective effects of sulphated Polysaccharides from *Codium edule* P.C Silva against UV-B induced Matrix metalloproteinase −1 production and skin damage. Int J Pharmacogn Phytochem Res, 2019;11(2):60-9.
7. Stockert JC, Horobin RW, Colombo LL, Blázquez-Castro A., Tetrazolium salts and formazan products in Cell Biology: Viability assessment, fluorescence imaging, and labeling perspectives. Acta Histochem, 2018;120(3):159-67. doi: 10.1016/j.acthis.2018.02.005, PMID 29496266.
8. Gabbiani G, Gabbiani F, Heimark RL, Schwartz SM, Organization of actin cytoskeleton during early endothelial regeneration *in vitro*. J Cell Sci. ,1984 ;66:39-50. doi: 10.1242/jcs.66.1.39, PMID 6540272
9. Li Weida, Wang Koi, Jiang Nanfrang, Liu Xialei,Antioxidant and antihyperlipidemic activities of purified polysaccharides from *Ulva pertusa*. J Appl Phycol, 2018;30:2619-27.
10. Yu seok Song, Hailan Li, Marie Carmel Balcos, Hye-young yun, Fucoidan promotes the reconstruction of the skin equivalents, Korean Journal of Physiology and pharmacology, 2014, 18(4),327-331.
11. Hee Jung Moon, Sang Ho Lee , Byeng Chul Yu, Man Joong Jeon, Seok Hoon Jeong, Valentin A Stonik, Tatyana N Zvyagintseva, Svetlana P Ermakova, Yong Hwan Lee, Effect of *Costaria costata* fucoidan on expression of matrix metalloproteinase-1 promoter, mRNA, and protein, Nat Prod, 2009, Europ J. of dermatol, 19(2):129-134.
12. Nie W, Deters AM, Tamarind Seed Xyloglucans Promote Proliferation and Migration of Human Skin Cells through Internalization via Stimulation of proliferative Signal Transduction Pathways, Hindawi Publishing Corporation Dermatology Research and Practice Volume; 2013, 14 p: Article ID 359756.
13. Slim Smaoui, Hajer Ben Hlima, Raoudha Jarraya, Nozha Grati Kamoun, Raoudha Ellouze, Mohamed Damak, Cosmetic emulsion from virgin olive oil: Formulation and bio-physical evaluation,Afr J Biotechnol, 2012,11(34):8417-24.
14. Avish D Maru, Swaroop R Lahoti, Formulation and evaluation of moisturizing cream containing sunflower wax. Int J Pharm Pharm Sci. ,2018;10(11):54-9.
15. Brode GL. New York: Plenum press; (Cosmetics and pharmaceutical applications of polymers., 1991, pp:105-15.
16. Mayuree Kanlayavattanakul and Nattaya Lourith, , Biopolysacchrides for the skin hydrating cosmetics, Springer International Publishing, 2015, 72(10):1731-4:1868-90
17. Jae Woo Choi, Soon Hyo Kwon, Chang-Hun Huh, Kyoung Chan Park, the influence of skin visco elasticity, hydrating level and aging on the formation of wrinkles: A comprehensive and objective approach, Skin Research and Technology, 2013, 19(1).