Pharmacogn. Commn. 2017; 7(4):152-157
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogcommn.org

Original Article

Correlating the Anti – Aging Activity with the Bioactive Profile of *Chlorella emersonii* KJ725233; its Toxicological Studies for a Potential use in Cosmeceuticals

Sneha Sunil Sawant, Varsha Kelkar Mane*
Department of Biotechnology, University of Mumbai, Kalina, Mumbai, Maharashtra, INDIA.

ABSTRACT

Background: Microalgae due to an array of its antioxidant, antimicrobial and anti-inflammatory bioactives have been changing the cosmeceutical market. One such bioactive power pack is *Chlorella emersonii* KJ725233, a novel, non-fastidious freshwater microalga with an inherently higher antioxidant potential. **Objective:** The present study evaluates its potential use as a cosmeceutical ingredient with arrays of biochemical assays, toxicity studies and GC-HRMS profiling. **Materials and Methods:** *In vitro* proteinase inhibition activity of the methanolic extract of *C.emersonii* KJ725233 was evaluated using SAAANA, sodium hyaluronate and bovine collagen as substrates respectively. The radical scavenging potential was determined by DPPH assay and was correlated to the total flavonoid content evaluated by aluminium trichloride method. The bioactive composition was evaluated by GC – HRMS analysis. Eye irritation potential and sulforhodamine B assay were carried out using 3T3 fibroblasts cells to ascertain its non-toxic nature. **Results:** The methanolic extract of *C.emersonii* KJ725233 exhibited a potent elastase inhibition of 92.04±2.27% at a concentration of 1000 µg/mL. This was qualitatively established by the presence of phytol, its isomer and other anti-inflammatory molecules as revealed by GC HRMS profile. Its radical scavenging potential with an IC_{50} of 5.398±0.138 mg/mL exhibited a correlation coefficient of 0.9977 with its total flavonoid content. Toxicological studies on 3T3 fibroblast cell lines revealed its non – toxic as well as non – eye irritant nature. **Conclusion:** Its potential anti-aging and antioxidant activity coupled with its non-toxicity thus puts this microalga in the spot light for use in cosmeceutical industry.

Key words: *Chlorella emersonii* KJ725233, Anti-aging, Anti-oxidant, Anti-inflammatory, Bioactives, Cosmeceuticals.

Correspondence:
Dr. Varsha Kelkar Mane,
Department of Biotechnology, University of Mumbai, Kalina,
Mumbai, Maharashtra, INDIA.
Phone no: 022-26526053
E-mail: drvkelkar@mu.ac.in
DOI : 10.5530/pc.2017.4.22

INTRODUCTION

There exists a balance between the synthesis and degradation of the matrix proteins (collagen, elastin, glycosaminoglycans (GAGs) like hyaluronic acid) in healthy, radiant skin. This is maintained by degradation of the redundant matrix by the corresponding proteinases coupled with its replacement by continual synthesis. However, both chronological as well as photo – aging disrupts this balance by coupling the upregulation of the proteinases, thus enhancing degradation of the proteins, with downregulation of their synthesis. This disparity can be improved by either stimulating the synthesis of the proteins or by altering their breakdown by proteinases.[1,2,3,4,5,6,7,8] In addition to maintaining the skin integrity, these proteinases also play a vital role in inflammatory responses to photo – aging.[9,10]

For this purpose, a variety of skin rejuvenation formulations containing ethanolamines, sodium lauryl sulphates, polypeptides or oligopeptides (copper peptides, palmitoyl pentapeptides), tretinoin etc. are available that are either aimed at improving the synthesis of the matrix proteins (except elastin) or inhibiting the proteinases.[11,12,13,14] However, the inadequate efficacies of these formulations due to the variations in skin types coupled with an increase in consumer suspicion about their adverse effects (allergic contact dermatitis, irritant contact dermatitis, phototoxic and photoallergic reactions) eventually led to an incessant search for novel bioactives to replace their synthetic counterparts in the cosmetic formulations.[11] The green cosmetic industry therefore, has been for long, leveraging on the bioactive power packs - algae as moisturizing as well as thickening agents.[15] The secondary metabolites of microalgae with antioxidant, anticancer, antibacterial, anti-inflammatory, anti – aging, anti-allergic properties[16,17] have made them an attractive alternative to their synthetic equivalents for cosmeceutical formulations. According to one of our previous studies, methanolic extracts of *Chlorella emersonii* KJ725233, an isolate from western Maharashtra, exhibited higher antioxidant capacity as compared to other solvent extracts[18] and hence was subjected to an array of anti - aging and toxicity tests.

The anti – aging potential was evaluated for elastase, hyaluronidase, collagenase activity inhibition assays. Radical scavenging potential was determined by 1,1-diphenyl-2-picrylhydrazyl radical scavenging assay along with its cytotoxicity by eye irritation potential and sulforhodamine B assay using 3T3 fibroblast cell line. Being a novel isolate it was also subjected to GC – HRMS analysis so as to identify its possible anti-aging constituents.

MATERIALS AND METHODS

Materials

BG – 11, calcium chloride and DPPH were purchased from HiMedia, India. Methanol, dimethyl sulfoxide (DMSO) and aluminium trichloride of AR grade were obtained from S D Fine-chem ltd., India. SAAANA, porcine pancreatic elastase, bovine hyaluronidase, p- dimethyl amino benzaldehyde, bovine collagen, N – [Tris(hydroxymethyl)methyl] – 2 – aminoethanesulfonic acid (TES buffer), ninhydrin, citric acid, sulforhodamine B, tween 20, triton X – 100 and sodium lauryl sulphate of molecular biology grade were procured from Sigma.

Statistics

All the tests have been performed in triplicate and the values are expressed as mean ± SD.

Culturing of *Chlorella emersonii* KJ725233 and preparation of its extract

Chlorella emersonii KJ725233 was grown in BG – 11 medium in 12 h: 12 h light: dark conditions with 12 h of aeration at 32±1°C. After 20 d of incubation, the biomass was harvested by centrifugation at 5000 rpm for 15 min and dried at 60°C for 24 h. The dried biomass was suspended in methanol at a concentration of 0.1 mg/mL and sonicated for 30 min. The methanolic suspension was then centrifuged at 5000 rpm for 15 min and the supernatant transferred to a crucible for drying. The mass was subjected thrice to sequential extractions and all the supernatants were pooled. 1 µL of this extract was subjected to for its metabolite composition by GC-HRMS (Agilent Technologies, USA) analysis whereas the remaining extract was allowed to dry at 28± 2°C and then reconstituted in DMSO so as to achieve a concentration of 1 mg/mL.[18]

Anti – aging activity

The anti – aging activity was determined in terms of its *in vitro* elastase, hyaluronidase and collagenase inhibition activity.

The anti – elastase activity was determined spectrophotometrically by using SAAANA substrate as described by Thring *et al.*[7] Porcine pancreatic elastase enzyme was incubated with varying concentrations of the extract (100 µg/mL, 500 µg/mL, 1000 µg/mL). After 15 min of incubation the substrate was added and the reaction was further incubated for 30 min at 25°C. The absorbance was read at 410 nm. Epigallocatechin was assayed as a positive control.

Anti - Hyaluronidase activity was spectrophotometrically determined by measuring the amount of N-acetyl glucosamine produced by the action of bovine hyaluronidase on sodium hyaluronate as earlier reported by Lee *et al.*[19] Bovine hyaluronidase was added to varying concentrations of the extract (100 µg/mL, 500 µg/mL, 1000 µg/mL) and incubated at 37°C for 20 min. Hyaluronidase was activated by adding 12.5 mM calcium chloride to the reaction mixture and was further incubated at 37°C for 20 min. After the incubation, 1.2 mg/mL sodium hyaluronate was added and incubated at 100°C in a water bath for 3 min. The reaction mixture was allowed to cool at room temperature, after which 1.5 mL of p-dimethyl amino benzaldehyde was added to it and incubated at 37°C for 20 min. The absorbance was measured at 585 nm. Gallic acid was used as a positive control.

Anti-collagenase activity was determined as earlier described by Mandl *et al* and Moore *et al.*[20,21] Collagenase was incubated with varying concentrations of extract at 37°C for 30 min. To 25 mg of bovine collagen, 0.05 M TES buffer was added and incubated at 37°C for 15 min. After incubation, 0.1 mL of the treated collagenase was added to the collagen containing mixture and allowed to react. After 5 h, 0.2 mL of this reaction mixture was transferred to tubes containing 1.0 mL of ninhydrin-citric acid mixture and was incubated in a boiling water bath for 20 min. After cooling 5 mL of 50% n-propanol was added to it and allowed to stand for 15 min. The absorbance was measured at 600 nm.. Catechin was used as a positive control.

Radical scavenging activity

The radical scavenging potential was determined by using DPPH as described by Bendaoud *et al.*[22] To 150 µL of extract concentrations ranging from 0.2 – 1 mg/mL, an equal volume of 0.2 mM methanolic DPPH was added. The reaction mixture was incubated in the dark at 28±2°C for 30 min.

The absorbance was measured at 517 nm. Percent inhibition was calculated by the following formula:

$$\text{Percent Inhibition} = \frac{(\text{Absorbance of blank} - \text{Absorbance of test})}{\text{Absorbance of blank}} * 100$$

A graph of percent inhibition versus the extract concentration was plotted and IC_{50} was determined.

Total flavonoid content

The flavonoid content of the methanolic extract of *C.emersonii* KJ725233 was evaluated by the aluminium trichloride method.[23] To 150 µL of the extract, an equal volume of 2% aluminium trichloride was added and incubated in the dark at 28±2°C for 30 min. The absorbance was read at 470 nm. Quercetin was used as a standard and the total flavonoid content was expressed as mg QE/g dried biomass.

Identification of bioactives by gas chromatography – high resolution mass spectrometry

GC- HRMS analysis of all the methanolic extracts were carried out using GC (Agilent Technologies, USA) equipped with Accutoff MS. Compounds were separated on HP-5 MS capillary column having 5% phenyl polysiloxane as stationary phase, column length 30 m , internal diameter 0.32 mm and film thickness 0.25 µm . 1 µL of the methanolic extracts were injected in the split ratio of 10:1, the injector and transfer line temperature was 250°C and 260°C while the ion source temperature was 200°C. Oven temperature programmed from 80 to 280°C at 10°C min^{-1}; flow rate of carrier gas helium was 1 mL min^{-1}. Compounds were identified by comparing their retention times and mass fragmentation patterns with those of the standards at the NIST library.

Cytotoxicity and Irritation assessment

Cytotoxicity and irritation assessment was determined by the sulforhodamine B assay using 3T3 Swiss albino mouse embryonic fibroblast cell lines. Cells were seeded into a 96 well plate and incubated at 37 °C for 24h in 5% CO_2 95% air with 60 – 70% humidity. Varying concentrations of the extracts were added to the cells and incubated for another 24 h. After incubation, the cells were observed under microscope, fixed, washed and dried. The cells were then stained with sulforhodamine B stain, washed and dried. The stain was further solubilized in Tris buffer and the absorbance was read at 530 nm. Tween 20, sodium lauryl sulphate and Triton – X 100 were used as reference standards.[24,25]

RESULTS AND DISCUSSIONS

Anti – aging activity

Aging leads to a decrease in the synthesis of skin structural proteins like elastin, collagen and hyaluronic acid resulting in the loss of skin elasticity, laxity, integrity eventually leading to the visible signs of aging.[1,8] The age dependent downregulation of skin elastic properties is not only attributed to a decreased synthesis of these proteins, but also to an upregulation of the corresponding proteinases.[26,27] Elastase, a member of the chymotrypsin family of serine proteinases is mainly responsible for the degradation of elastin.[7] Hyaluronic acid is degraded by another serine proteinase – hyaluronidase whereas collagenase a zinc containing matrix metalloproteinase hydrolyzes collagen.[7,8] The activity of these proteinases is not only stimulated with chronological aging but also with a prolonged UV – exposure.[7] Therefore, the prime mover for anti-aging strategy to achieve healthy, smooth, blemish free and resilient skin is by means of controlling the degradation of the skin structural constituents with regulation of proteinase activity.[12,28]

With its intrinsically high antioxidant activity, *C.emersonii* KJ725233 was taken up for assessing its potential as a proteinase inhibitor in the present study. The anti - elastase activity of its methanolic extract was evaluated using SAAANA as a substrate and the percent enzyme inhibition was correlated to the amount of p – nitroaniline formed.[7] Significant elastase inhibition of 92.04±2.27% was observed at 1000 µg/mL (Figure 1) with

an IC_{50} of 0.49±0.01 g/mL (Table 1). The IC_{50} value is inversely proportional to the enzyme inhibition potential. Therefore, a lower IC_{50} signifies a higher anti – proteinase activity. Although the IC_{50} value was found to be equivalent to that of *C.emersonii* 11N i.e 0.42±0.05 mg/mL, it was lower than that of 0.503±0.06 mg/mL for *Tetraselmis suecica.*[28,29]

Although the methanolic extract of *C.emersonii* KJ725233 exhibited a modest anti-hyaluronidase activity of 39.32±0.92% at 1000 µg/mL (Table1); its IC_{50} of 1.58±0.06 mg/mL (Figure 1) was fivefold lower than that of *Dunaliella tertiolecta* (5.542±0.12 mg/mL), *Tetraselmis suecica* (5.907±0.26 mg/mL) and four fold lower than that of *Nannochloropsis* species (4.202±0.33 mg/mL) as previously reported.[28]

The methanolic extract of *C.emersonii* KJ725233 exhibited a significant anti – elastase and a modest anti – hyaluronidase activity however, its anti – collagenase potential was found to be less significant with a percent inhibition of 20.33±2.90% at 1000 µg/mL (Table 1) with an IC_{50} of 2.50±0.03 mg/mL (Figure 1). However, the present study is one of the first to report anti-collagenase activity of any microalga, signifying its potential in cosmeceuticals.

Radical scavenging potential and total flavonoid content

Repetitive and prolonged UV exposure leads to an over production of the ROS which induces oxidative cellular stress and promotes genetic alterations thereby affecting matrix proteins structure as well as functions.[1] ROS induces the over expression of the matrix metalloproteinases and serine proteinases in the fibroblasts therefore causing the remodelling of skin extracellular matrix, resulting in skin damage.[28,29,30] To combat this oxidative stress, there is need to scavenge these free radicals. The methanolic extract of *C.emersonii* KJ725233 was thus evaluated for its radical scavenging potential by the DPPH method (Figure 2). A graph of percent inhibition versus extract concentration was plotted and the IC_{50} value was found to be 5.40±0.14 mg/mL. The IC_{50} of the methanolic extract of *C.emersonii* KJ725233 was found to be lower than that reported for the commercial Sun chlorella powder of *C. pyrenoidosa* i.e 9.62 mg/mL as previously reported.[31]

DPPH has been widely used for measuring the antioxidant status of phenolic compounds like flavonoids, since they have the ability to donate their hydrogen and thus function as strong free radical scavengers.[32,33] Flavonoids are also known to bind metalloenzymes like collagenase via hydrogen as well as hydrophobic interactions, thereby inducing conformational changes and inhibiting it from binding to the substrate.[7,34] However, flavonoids not only inhibit collagenase, but also are potent elastase inhibitors.[34]

The total flavonoid content of the methanolic extract of *C.emersonii* KJ725233 was determined by the aluminium trichloride method and was found to be 14.26±1.31 mg QE/g dried biomass i.e 158.09±0.23 mg QE/g dried extract which is 20.54% higher as compared to that of 131.15 mg QE/g dried extract of *C.vulgaris.*[35] The coefficient of correlation for DPPH radical scavenging potential and total flavonoid content was found to be 0.9971 signifying the contribution of flavonoids to the radical scavenging potential of the methanolic extract of *C.emersonii* KJ725233.

Identification of bioactives by GC-HRMS

Since the methanolic extract of *C.emersonii* KJ725233 exhibited proteinase inhibition as well as radical scavenging potential it was thought worthwhile to identify the bioactives contributing to this activity. The methanolic extract was therefore, subjected to GC-HRMS analysis and the compounds were identified by comparing the mass fragmentation pattern with those in the NIST library as seen in (Table 2).

Phytol - a major contributor of the methanolic extract and its isomer 3,7,11,15 – tetramethyl – 2 – hexadecen – 1 - ol are reported for their antioxidant as well as anti – inflammatory activities.[36,37] The hydrocarbon – hexadecane another major contributor of the methanolic extract after phytol is known for its antioxidant as well as anti-inflammatory activities.[38,39] 9,12 – octadecadienoyl chloride is reported as an anti – inflammatory, cancer preventive, anti – acne, anti – eczema, anti – histaminic etc. whereas 3 –trifluoroacetoxypentadecane is ant oxidative as well as anti-inflammatory in nature.[40,41,42]

The presence of several anti-inflammatory molecules in the methanolic extract may contribute to the anti-proteinase activity of *C.emersonii* KJ725233 since the proteinases elastase, hyaluronidase and collagenase are known to be involved in the inflammatory responses.

Cytotoxicity and Irritation assessment

Although the efficiency of bioactives in particular therapeutic approaches is imperative, its safety and absence of adverse cellular side effects is of utmost concern. Hence, along with cytotoxicity, assessment of irritation potential is a vital part of toxicology studies of new materials prior to consumer use.

Table 1: Percent inhibition of the proteinases at varying concentrations of the extract.

Concentration of the extract (µg/mL)	Percent inhibition Elastase	Percent inhibition Hyaluronidase	Percent inhibition Collagenase
100	42.49±1.74	11.65±1.42	0.0
500	65.22±2.43	20.87±1.63	0.0
1000	92.04±2.27	39.32±0.92	20.33±2.90

Data is represented as mean±SD. p value<0.05 as compared to that of the control.

Table 2: Bioactives composition of the methanolic extract of *Chlorella emersonii* KJ725233 along with their retention times in minutes as well as area percentage.

Retention time (min)	Compound	% area
17.08	Hexadecane	25.1
19.0	3,7,11,15 – Tetramethyl – 2 – hexadecen – 1 – ol	3.188
20.15	Oxirane, [(dodecyloxy) methyl]	5.8
20.41	Pentadecanoic acid, 14 – methyl - , methyl ester	1.747
23.65	9,12 – Octadecadienoyl chloride	2.01
23.75	9,12 – Octadecadienoyl chloride	3.11
24.03	Phytol	38.74
25.36	3 –Trifluoroacetoxypentadecane	7.529
29.05	3 –Trifluoroacetoxypentadecane	5.4
29.67	9,12 – Octadecadienoyl chloride	5.07
31.23	9 – Octadecanoic acid – phenylmethyl ester	2.26

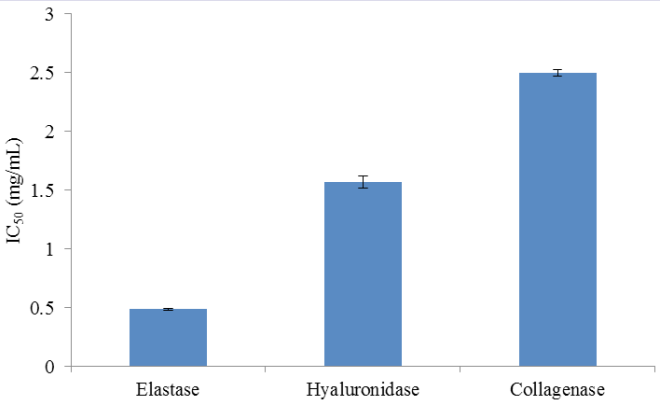


Figure 1: IC_{50} values for enzyme activity inhibition (Anti – Elastase: 0.49±0.1 mg/mL, Anti – Hyaluronidase: 1.58±0.06 mg/mL; Anti – Collagenase: 2.50±0.03 mg/mL).

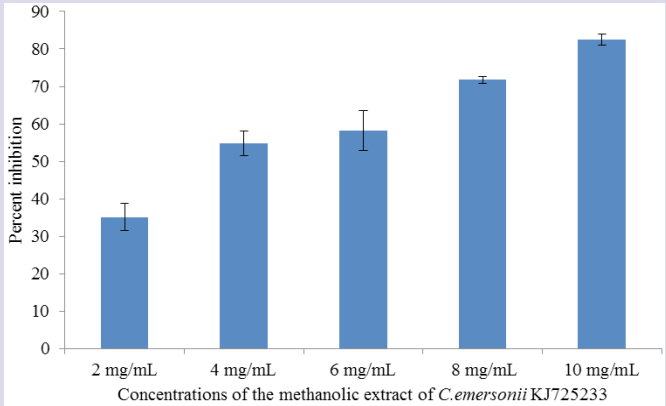


Figure 2: A graph of DPPH radical scavenging in terms of percent inhibition versus varying extract concentrations of methanolic extract of *C.emersonii* KJ725233.

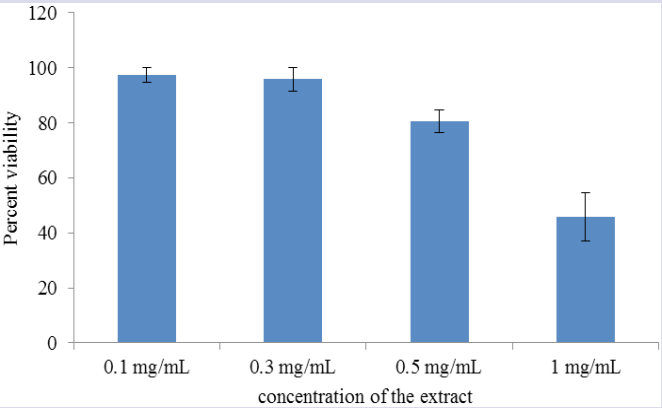


Figure 3: A graph of percent viability of 3T3 fibroblast cells after treatment with varying concentrations of the methanolic extract of *Chlorella emersonii* KJ725233.

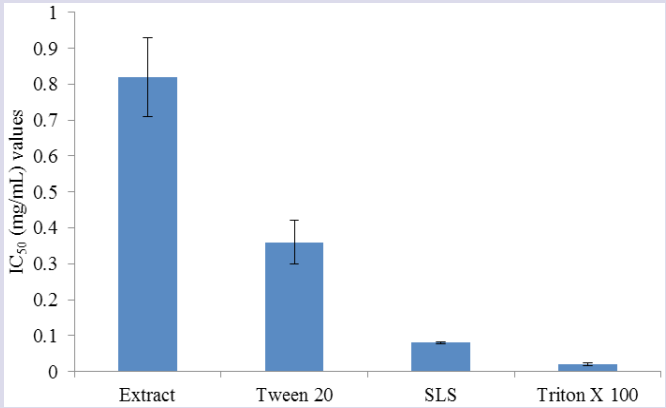


Figure 4: Comparison of the IC_{50} of the methanolic extract of *Chlorella emersonii* KJ725233 with those of the reference standards used - Tween 20, SLS and Triton X 100.

Therefore, to analyze the safety of the methanolic extract of *C.emersonii* KJ725233 for use as a probable anti-aging agent its cytotoxicity was studied in terms of percent viability of the 3T3 fibroblast cell line (Figure 3) by sulforhodamine B assay.

By plotting a graph of percent viability versus extract concentration the IC_{50} (Figure 2) was found to be 0.82±0.11 mg/mL. Thus the methanolic extract was found to exhibit cytotoxicity at concentration greater than 0.82±0.11 mg/mL.

The eye irritation potential of the extract was determined in terms of the IC_{50} values derived from the dose response curves were graded in comparison with those of the reference standards (Figure 4).

As per the Japanese Ministry of Healthcare and welfare guidelines for manufacturing of cosmetics and quasidrugs,[25] comparing the IC_{50} value of the methanolic extract of *C.emersonii* KJ725233 with those of the reference standards, it can be concluded that it is practically a non – irritant since it has an IC_{50} higher than all the tested reference samples.

CONCLUSION

The presence of anti – inflammatory molecules like phytol, its isomer and hydrocarbons, as revealed from the GC-HRMS analysis are known to reduce the proteinases activity thus reducing inflammation of the dermis. The anti – aging properties of *Chlorella emersonii* KJ725233 thus stem from its ability to inhibit elastase, hyaluronidase, and collagenase and to scavenge free radicals which might aid in supporting skin tissue leading to skin rejuvenation. Due to a plethora of compounds exhibiting anti – aging, antioxidant, anti – inflammatory properties; *Chlorella emersonii* KJ725233 has a great potential as a source of bioactive for cosmeceuticals.

ACKNOWLEDGEMENT

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS USED

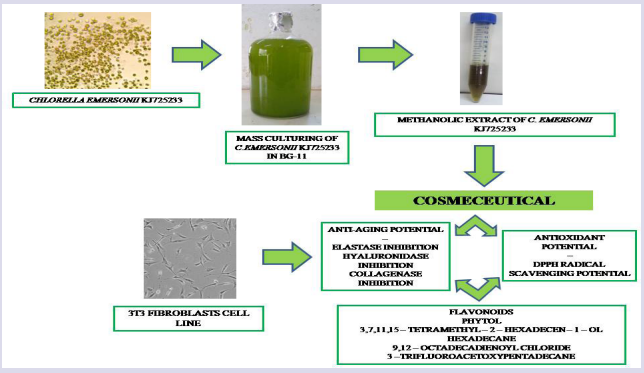
SAAANA: N – Succinyl – Ala – Ala – Ala – p – nitroanilide; **DPPH:** 1,1-Diphenyl-2-picrylhydrazyl; **GC – HRMS:** Gas Chromatography – High Resolution Mass Spectrometry.

REFERENCES

1. Ndlovu G, Fouche G, Tselanyane M, Cordier W, Steenkamp V. *In vitro* determination of the anti-aging potential of four southern African medicinal plants. BMC Complement Altern Med. 2013;13(1):304 – 10. http://dx.doi:10.1186/1472-6882-13-304
2. Maity N, Nema NK, Abedy S, Sarkar BK, Mukherjee PK. Exploring *Tagetes erecta* Linn flower for the elastase, hyaluronidase and MMP-1 inhibitory activity. J Ethnopharmacol. 2011;137(3):1300–5. http://dx.doi: 10.1016/j.jep.2011.07.064
3. Kurtz A, Oh S-J. Age related changes of the extracellular matrix and stem cell maintenance. Prev Med. 2012;54:S50–6. http://dx.doi: 10.1016/j.ypmed.2012.01.003

4. Fulop T, Khalil A, Larbi A. The role of elastin peptides in modulating the immune response in aging and age-related diseases. Pathol Biol. 2012;60(1):28–33. http://dx.doi: 10.1016/j.patbio.2011.10.006
5. Wary KK, Thakker GD, Humtsoe JO, Yang J. Analysis of VEGF-responsive genes involved in the activation of endothelial cells. Mol Cancer. 2003;2(1):25–36. http://dx.doi: 10.1186/1476-4598-2-25
6. Losso JN, Munene CN, Bansode RR, Bawadi HA. Inhibition of matrixmetalloproteinase-1 activity by the soybean Bowman–Birk inhibitor. Biotechnol Lett. 2004,26(11):901–5. PMID:15269538
7. Thring TSA, Hili P, Naughton DP. Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants. BMC Complement Altern Med. 2009;9(1):27–37. http://dx.doi: 10.1186/1472-6882-9-27
8. Buhren BA, Schrumpf H, Hoff NP, Bolke E, Hilton S, Gerber PA. Hyaluronidase: from clinical applications to molecular and cellular mechanisms. Eur J Med Res. 2016;21:5. http://dx.doi: 10.1186/s40001-016-0201-5
9. Lehra KS, Kaur R, Sharma S, Kapoor A, Singh S. Anti – inflammatory agents from plants – Part III. Indian J Nat Prod Resour. 2014;5(2):121 – 8.
10. Lee SH, Sancheti S, Sancheti S, Seo S-Y. Potent antielastase and antityrosinase activities of *Astilbe chinensis*. Am J Pharmacol Toxicol. 2009;4(4):127-9. http://dx.doi : 10.3844/ajptsp.2009.127.129
11. Mukherjee PK, Maity N, Nema NK, Sarkar BK. Bioactive compounds from natural resources against skin aging. Phytomedicine. 2011;19(1):64–73. http://dx.doi: 10.1016/j.phymed.2011.10.003.
12. Gancevicience R, Liakou AI, Theodoridis A, Makrantonaki E, Zouboulis CC. Skin anti-aging strategies. Dermatoendocrinol. 2012;4(3):308–19. http://dx.doi: 10.4161/derm.22804
13. Robinson LR, Fitzgerald NC, Doughty DG, Dawes NC, Berge CA, Bissett DL. Topical palmitoyl pentapeptide provides improvement in photoaged facial skin. Int J Cosmet Sci. 2005;27(3):155 – 60. http://dx.doi: 10.1111/j.1467-2494.2005.00261.x
14. Schagen SK. Topical peptide treatment with effective anti – aging results. Cosmetics 2017;4(2):16. http://dx.doi:10.3390/cosmetics4020016
15. Wang H-MD, Chen C-C, Huynh P, Chang J-S. Exploring the potential of using algae in cosmetics. Bioresour Technol. 2015;184:355 – 62. https://doi.org/10.1016/j.biortech.2014.12.001
16. Abd EBH, El - Baroty GS. Healthy benefit of exploring microalgal bioactive substances. J Aq Sci. 2013;1(1):11-23. https://doi: 10.12691/jas-1-1-3
17. Priyadarshini I, Rath B. Commercial and industrial applications of microalgae. J Algal Bio Utln. 2012;3:89–100.
18. Sawant SS, Joshi AA, Bhagwat AM, Mane VK. Tapping the antioxidant potential of a novel isolate-*Chlorella emersonii* KJ725233. World J Pharm Res. 2014;3:726–39.
19. Lee KK, Cho JJ, Park EJ, Choi JD. Anti-elastase and anti-hyaluronidase of phenolic substances from *Areca catechu* as a new anti-ageing agent. Int J Cosmet Sci. 2001;23:341 – 6. https://doi: 10.1046/j.0412-5463.2001.00102.x
20. Mandl I, Zipper H, Ferguson LT. *Clostridium histolyticum* collagenase : its purification and properties. Arch Biochem Biophys. 1958;74(2):465 – 75. PMID: 13534676
21. Moore S, Stein WH. Photometric ninhydrin method for use in chromatography of amino acids. J Biol Chem. 1948;176:367–88.
22. Bendaoud H, Bouajila J, Rhouma A, Romdhane M. GC / MS analysis and antimicrobial and antioxidant activities of essential oil of *Eucalyptus radiate*. J Sci Food Agric. 2009;89(8):1292–7. https://doi: 10.1002/jsfa.3585
23. Colak N, Torun H, Gruz J, Strnad M, Hermosín-Gutiérrez I, Hayirlioglu-Ayaz S,*et al*. Bog bilberry phenolics, antioxidant capacity and nutrient profile. Food Chem. 2016;201:339 – 49. https://doi: 10.1016/j.foodchem.2016.01.062
24. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D *et al*. New colorimetric cytotoxicity assay for anti-cancer drug screening. J Natl Cancer Inst. 1990;82(13):1107 -12. PMID: 2359136
25. The Guidebook on application for the manufacturing of cosmetics and quasidrugs, Third Ed., Supervised by the Pharmaceuticals and Cosmetics Division, Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare, Yakuji Nippo Co, Ltd., 1996.
26. Thomas NV, Kim S-K. Beneficial effects of marine algal compounds in cosmeceuticals. Mar Drugs 2013;11(1):146 – 64. https://doi: 10.3390/md11010146
27. Imokawa G, Ishida K. Biological mechanisms underlying the ultraviolet radiation-induced formation of skin wrinkling and sagging I : Reduced skin elasticity, highly associated with enhanced dermal elastase activity, triggers wrinkling and sagging. Int J Mol Sci. 2015;16(4):7753-75. https:// doi: 10.3390/ijms16047753
28. Norzagaray - Valenzuela CD, Valdez – Ortiz A, Shelton LM, Jimenez – Edeza M, Rivera – Lopez J, Valdez – Flores MA, *et al*. Residual biomasses and protein hydrolysates of three green microalgae species exhibit antioxidant and anti - aging activity. J Appl Phycol. 2017;29(1):189 –98.
29. Chatzikonstantinou M, Kalliampakou A, Gatzogia M, Flemetakis E, Katharios P, Labrou NE. Comparative analyses and evaluation of the cosmeceutical potential of selected *Chlorella* strains. J Appl Phycol. 2017;29(1):179 – 88. https:// doi: 10.3390/ijms16047753
30. Wahab NA, Rahman RA, Ismail A, Mustafa S, Hashim P. Assessment of antioxidant capacity, anti-collagenase and anti-elastase assays of Malaysian unfermented cocoa bean for cosmetic application. Nat Prod Chem Res. 2014;2:132.
31. Geetha BJ, Navasakthi R, Padmini E. Investigation of antioxidant capacity and phytochemical composition of sun *chlorella* – an *in vitro* study. J Aquac Res. Development 2010; 1: 104. https:// doi: 10.4172/2329-6836.1000132
32. Apak R, Guclu K, Demirata B, Ozyurek M, Celik SE, Bektasoglu B *et al*. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. Molecules. 2007;12(7):1496–547. PMID: 17909504
33. Ahmed D, Fatima K, Saeed R. Analysis of Phenolic and Flavonoid Contents, and the Anti-Oxidative Potential and Lipid Peroxidation Inhibitory Activity of Methanolic Extract of *Carissa opaca* Roots and Its Fractions in Different Solvents. Antiox. 2014;3(4): 671–83. https:// doi:10.3390/antiox3040671
34. Hong W-H, Jung EY, Noh DO, Suh HJ. Physiological effects of formulation containing tannase – converted green tea extract on skin care : physical stability, collagenase, elastase and tyrosinase activities. Integr Med Res. 2014;3(1):25–33. https://doi.org/10.1016/j.imr.2013.12.003
35. Jayshree A, Jayshree S, Thangaraju N. *Chlorella vulgaris* and *Chlamydomonas reinhardtii* : effective antioxidant, antibacterial and anticancer mediators. Indian J Pharm Sci. 2016;78(5):575–81. https://doi:10.4172/pharmaceutical-sciences.1000155
36. Venkata RB, Samuel LA, Pardha SM, Narashimha RB, Naga VKA, Sudhakar M *et al*. Antibacterial, antioxidant and GC-MS analysis of *Eupatorium odoratum*. Asian J Pharm Clin Res. 2012;5(2):99-106.
37. Krishnamoorthy K, Subramaniam P. Phytochemical profiling of leaf, stem and tuber parts of *Solena amplexicaulis* (Lam.) *Gandhi* using GC-MS. Int Sch Res Not. 2014;1-13. http://dx.doi.org/10.1155/2014/567409
38. Yogeswari S, Ramalakshmi S, Neelavathy R, Muthumary J. Identification and comparative studies of different volatile fractions from *Monochaetia kansensis* by GCMS. GlobJ Pharmacol. 2012;6(2):65-71.
39. Peters RF, White AM. The relationship between cyclic adenosine 3',5' - monophosphate and biochemical events in the rat skin after the induction of epidermal hyperplasia using hexadecane. Br J Dermatol. 1978; 98:301-14. http://dx.doi: 10.1111/j.1365-2133.1978.tb06157.x
40. Omorowa OG, Idu M, Oghale O – U. GC – MS analysis of the aqueous extract of *Buchholzia coriacea Engl* (Capparidaceae) seeds. Int J Life Sci Pharma Res. 2015;5(3):26-32.
41. Hussein AO, Mohammed GJ, Hadi MY, Hameed IH. Phytochemical screening of methanolic dried galls extract of *Quercus infectoria* using gas chromatography – mass spectrometry (GC – MS) and Fourier transform – infrared (FT – IR). J Pharmacognosy Phytother. 2016;8(3):49-59. http://dx.doi: 10.5897/jpp2015.0368
42. Hussein HM, Hameed IH, Ibraheem AO. Antimicrobial activity and spectral chemical analysis of methanolic leaves extract of Adiantum capillus – veneris using GC – MS and FT – IR spectroscopy. Int J Pharmacog Phytochem Res. 2016;8(3):369-85.

PICTORIAL ABSTRACT



SUMMARY

- The methanolic extract of *C.emersonii* KJ725233 an antioxidant reserve exhibited a potent elastase inhibition potential.
- Additionally, it also demonstrated a substantial hyaluronidase as well as collagenase inhibition.
- This anti-proteinase inhibition potential can be attributed to the presence of anti-inflammatory molecules in the methanolic extract of *C.emersonii* KJ725233 as confirmed by GC-HRMS analysis.
- A compelling radical scavenging as well as elastase, collagenase inhibition may be accredited to the presence of flavonoids.
- The antioxidant, anti-proteinase potential of methanolic extract of *C.emersonii* KJ725233 thus projects this alga in the limelight as an anti-aging resource.

ABOUT AUTHORS

Ms. Sneha Sunil Sawant: Is a Research Scholar. She is currently working on the bio actives from freshwater microalgae; their metabolic responses under altered growth conditions as well as their remediation potential.

Dr. Varsha Kelkar Mane: Is a Senior Assistant Professor. She has been working actively in varied fields encompassing nanotechnology, algal bio actives, small molecular weight antimicrobial proteins and archival restoration.