VOLUME - 12, ISSUE - 04, APRIL - 2023 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Biotechnology

PHYCOBILLIPROTEINS AND ITS ECONOMIC VALUES – AN OVERVIEW

R Devika*

Department of Biotechnology, AarupadaiVeedu Institute of Technology, Vinayaka Missions Research Foundation, Paiyanoor 603104
*Corresponding Author

AK Ramya

Department of Microbiology, Aarupadai Veedu Medical College and Hospital, Vinayaka Missions Research Foundation, Pondicherry

ABSTRACT Marine ecosystem plays an important role in the country's economy for its valuable resources and top listed in the productivity environment. The plants and algae are known for its medicinal values and they are used in the primary health care and the bioactive compounds are proven to have therapeutic values. The special properties of phycobiliproteins are photosensitizer, chemical and thermal stability at varied pH. Phycobiliproteins extracted from macro and micro algae has wide applications in various industrial sectors like food, colourant, medicine, analytical sensing, pharmaceutical industries etc.

KEYWORDS : Seaweeds, bioactive compound, feedstock, phycoerythrin, phycobiliprotein, therapeutic value

INTRODUCTION

Our Indian subcontinent comprises of 7500 km coastline with 2.5 million km^2 Exclusive Economic Zone (EEZ) and they account for the intergral part of the economy of our country.The primary and secondary metabolites from marine organisms has become a fascinating subject and active research are recorded across the globe[1]. Exploration in Gulf of Mannar (Indian coast) recorded various marine resources which have highly potent applications in various industrial sectors[2].

Marine ecosystem are distinctive ecosystem functions such as much in three aspects like social, biological & economic significance and are reported as the most productive environment in the world[3-5]. Marine plants are an important part of the natural richness of developing countries and provide a significant contribution to the health care systems in those countries. They play a vital role in providing primary health care facilities and services to persons living in rural areas for traditional and modern medicines, they function as important therapeutic agents. The bioactive compounds from marine seaweeds and microalgae have recorded to have significant potential (active compounds) which is to be unexploited thoroughly[6-8].

Seaweeds And Its Bioactive Compounds

Few marine microalgae and seaweeds are exploited for many products in various sectors. *Gelidiumsequipedale* (red algae) is used for agar production[9], bioactive products obtained from plants and algae are used in food processing[10,11].

Microalgae possess unusual lipid compounds which are proposed to be biorefinery feedstock and added value for fine chemicals and fuels[12-15], lipids are used as aquaculture feeds[16], biodiesel production[17,18], supplement for human[19,20]. Though, agar extraction process from microalgae genetrate huge amount of by-products which are potential fertilizer[21]. *Gelidiumsequipedale* are rich in low molecules weight carbohydrates, proteins, mycosporine like amino acids (MAA), fatty acid, phenols, flavonoids etc. and proved to be potent nutritional value and posses biological activities like antioxidant, antimicrobial, anti inflammatory, cytotoxic etc[22-24].

Red algae (Rhodophyta) are rich with phycobiliproteins (PBPs) which are non toxic, water soluble, strong absorbance, fluorescence property, free radical scavenging, antioxidant etc[25-27]. PBPs are present in thylokoids membrane of seaweed's chloroplast which are very valuable in industries and has wide biotechnological applications[28]. The extraction process requires appropriate solvents and cell disruption methods[29].

Phycobiliprotein comprises of Phycoerythrin (R-PE), Allophycocyanin (APC), Phycocyanin (R_PC) in which the Rhodaphyceae is rich with R-PE and whereas cyanophyceae registered C-PC[30,31]. R-PE was purified from ten different seaweeds from the coast of Kilakarai, Gulf of Mannar[32] and it is listed in the Table I.

Phycobiliproteins are photosynthetic light harvesting andwater soluble proteins present in red, cryptomonads, cyanelles and cyanobacteria (covalently bound via cysteine amnio acid chromophores known as phycobilin – an open chain chromophore [33-35]. These proteins allows the transfer of light which cannot be used in photosynthesic mechanism (Chlorophyll a) and maintains the survival of the organisms even at low light[36] and the phycobilisome reacts as energetic funnel to reaction centers[37]. The phycobiliprotein is composed of α and β subunits with 2 or 3 chromophores (Phycoerythrobilin - PEB)[38,39], phycocyanobilin (PCB) and phycobiliviolin (PVB)[38]. Phycobiliproteins differ in amino acid sequences and divided into four types as phycoerythrin (PE), Phycoethyrocyanin, allophycocyanin lsted in the Table II[39,40].

Because of the high solubility and stability [41] nature of phycobiliprotein are used in various industrial sectors such as additives in pharmaceutical formulation[42], fluorescent label probe[43], colouringagent[44], as anti oxidant and anti cancer[45-47].

Methods Of Extraction And Quantification

Red algae collected should be subjected to repeated washing with salt water (35% w/v) to remove the contaminants. Various extraction processes were reported such as maceration, ultrasound assisted, extraction and high pressure assisted extraction[48,49].

Maceration

The algal biomass (100 mg)has to be suspended in phosphate buffer concentration ($0.01 M < C < 1 M$, pH 6.8) at different biomass : buffer ratio (R) (Vbuffer = 0.5-5ml, $1.5<R<1:50$) and homogenized using magnetic stirrer or motor and pestle at room temperature at different duration (5 min<30 min). Even efficient phycoerthrin extraction was obtained using sodium phosphate buffer at pH 6.8[50,51].

Ultrasound Assisted Extration

Biomass along with buffer suspension can be placed in ultrasonic bath or sonicator with an ultrasonic probe (ultrasonic pulse on & off 30/20S) at different time intervals. The sample is cooled in an ice bath to avoid overheating[51].

High Pressure Assisted Extraction

Biomass in 0.1M buffer suspension is placed in vacuum sealing bags and extraction has to be carried out at different time intervals (5min < 12 < 30 min) at different pressures (0.1 MPa < P < 600 MPa).

Phycobiliprotein Quantification

R Phycoerythrin (PE) and R- Phycocyanin (PC) quatification studies can be performed using Bear and Eshel, 1985 equations and the absorption spectra can be measured between 200-900 nm using UV-VIS spectrometer

PE (PE/g) = (A564 – A592) - (A455-A592) x 0.2} x 0.12

PC (PC/g) = (A618 – A645) – (A592 – A645) x 0.5} x 0.15

Purity index of the extract can be determined by Mensi and Romdhane, 2014.

PI = A564 / A 230 (564 for PE and A280 for total protein)

Response Surface Methodology (RSM)

A statistical model for optimization studies and evaluation of variable effects of PE can be determined by RSM yield from maceration and ultrasonic waves.

Extraction Of Phycobiliproteins From Microalgae

The economic value and its broad range of applications of phycobiliproteins involve efficient extraction and purification techniques such as cell disruption, pigment release, extraction and purification[52]. The cell disruption and release of pigments are maceration, ultrasound, microwave and freeze thraw under mechanical treatments. Other extraction processes are solid –liquid extraction (Specific solvents) enzymatic hydrolysis or combination of mechanical and chemical treatments[53]. Through maceration process *Gracilaria gracilis* yielded 3.53 mg g^{-1}[54], *Gelidiumpusillum* yielded 1.19 mg g^{-1}, respectively[55]. Through ultrasound assisted method the yield of phycobiliproteins from *Gracilaria .gracilis, Gelidium pusillum, Grateler paturuturu, Porphyridium cruentum* were 1.57 mg g^{-1}, 0.16 mg g^{-1}, 3.6 mg g^{-1} and 69 μg g^{-1}, respectively[54-56,12]. Through enzymatic hydrolysis Palmaria palmate and Gracilaria verrucosa yielded 3.28 g Kg^{-1}dw, 6,25 mg g^{-1} of pycobiliproteins, respectively[57,58]. *Spirulina maxima* yielded 0.8 mg ml^{-1} of Phycoeruthrin {PE} and 11.3 mg ml^{-1} of Phycocyanin {PC}, respectively through ulrasonication[59,60] and *Spirulina platensis* yielded 0.75 gL^{-1} of PC through combination of mechanical agitation and thermal heating[61]. Through microwave assisted process *Porphyridium cruentum* reported to yield 73.7 μg mg^{-1} and 34.8 μg mg^{-1} of PE and PC, respectively[62].

Purification Of Phycobiliproteins From Microalgae

Purification process involves combination of techniques and the purity index is expressed as A565 nm/ A280nm ratio[63] wherein for protein is 0.7 on food grade, 3.9 as reactive grade and > 4.0 as analytical grade[64]. Precipitation method of ammonium sulfate (two steps with 20% and 40%) before chromatographic separation removes amino acids[65] and achieved high PE purity of 5 in *Porphyridium marinum* algae and PE purity with 55% ammonium sulfate precipitation from red algae[66]. *Portierahoren emannti* and *Porphyridium cruentum* with 5.1[67]. Gel filteration with Sephacryl S 300 followed by anion exchange chromatography in *Lyngloyaar boricola* and *Synechococcus Sps.* had purity index of 5.2 and 3.4, respectively[68,69].

Expanded bed and anion exchange chromatography in *Pyropiahai tanensis* yielded 247.13 mg L^{-1} of PE with purity index[70]. Hydroxyapatite (as adsorbent) in chromatography and precipitation with ammonium sulfate yielded 5.5 PE and 5.1 PC from *Porphyray ezoensis*[71,72]. Purification and extraction recovery of PE and PC from microalgae is tabulated in the Table III.

Applications Of Phycobiliproteins

The phycobiliproteins because of its specific characteristics and properties like stability, bioactivity and biocompatibility they are employed/ utilized in various industrial sectors like food, textile, cosmetics and pharmaceutical sectors[72,73].

Phycobiliproteins In Analytic Sensing

Luminescent nanospore conjugated with phycocyanin is employed to detect mycelperoxides (α protein) that causes inflammation related diseases[74] and also Ochratoxin A and Zearalenone can be detected through quantitative fluorescence image analysis[75]. Phycoerythrin is used to detect transcription factor [76], toxic elements in effluent samples[77,78], mercury in the environment[79] etc., respectively. Phycoerythrin has recorded the maximum stability effect among the phycobiliprotein due to its ý subunits[80-82] when combining with sunlight harvesting solar optical temperature sensors [83].

Phycobiliproteins In Pharmaceutical Applications

Report on antioxidant activity with phycocyanin isolated from *Anabaena*Sps.proved to be efficient against DPPH (2,2 diphenyl-1-picrylhydrazl) and ABTs (2,2' azinobis-3-ethylbenzothiazoline-6-sulfonic acid) in rats where it attenuated the liver structural deformation caused by CCL_4 [84,85] and PE also effective against age related diseases in addition to antioxidant activator. R-PE has proved to be effective in anti inflammatory, immunomodulation, repair damaged mucosa, regulation of immune functions etc[86,87]. Phycobiliproteins behave photosensitizers[88] agents to destroy cancer cells and C-PE is an effective therapeutic agent against Alzheimers disease[89,90].

Phycobiliproteins In Food Applications

Phycobiliproteins act as natural food colorants because of its high stability and wide range of pH (4-10)[91,92]. Phycoerythrin is a natural red colourant, allophycocyanin and phycocyanin are usedin commercial devices such as Lehar soda, 7'up and TATA mineral water and it is proved to retain the colour for 30 days. Phycobiliproteins along with addition of acid/ salt / sucrose prevents denaturation denaturation and therefore add as preservatives in production of citric acid, calcium chloride, sodium chloride and sucrose[93]. Thermokinetic stability of PC and PE extracted from *Nostoc*Sps. has proved to be citric acid, ascorbic acid and calcium chloride[94]. PE and PC isolated from *Atacama* cyanobacteria recorded chemical stability at pH 5-8 at 50^0C [95,96].

CONCLUSION

The use of PBP has numerous applications in the current state of science. These skills aid in the development of numerous economic sectors, including as the food, colourant, pharmaceutical, and analytical sensing industries.The special properties of phycobiliproteins are photosensitizer, chemical and thermal stability at varied pH. Future developments in coastal seas and oceans, along with the development of red micro- and macroalgae marine cultures, should give rise to fresh stimuli for this.

Table I: Phycobiliprotein, Total Protein content from red algae

Red Algae	Phycobiliproteins (mg/g)			Total protein (%m/m)
	R-PE	R-PC	R-APC	
Portierio hornemanni	1.232	0.20	0.061	1.013
Hypneaesperi	0.416	0.18	0.046	0.461
Gelidiellaacerosa	0.545	0.19	0.062	0.928
Acanthophoraspicifera	0.421	0.34	0.121	1.061
Sarconemofiliforme	0.397	0.19	0.156	0.37
Laurensiapapillosa	0.182	0.36	0.043	0.981
Gelidiumpusilum	0.403	0.18	0.085	0.444
Gelidiumsalicornia	0.573	0.23	0.074	0.601
Acanthophoraspicifera	0.421	0.34	0.121	1-061

Table II: Characteristics features of types of phyco biliproteins

Types of Phycobiliproteins	Chemical Structure	Molecular weight	Absorption spectrum (nm)
Phycoerythrin	(∞β)6 Ycomplex	240	490-570
Phycoerthrocyanin	(∞β)	-	560-600
Phycocyanin	(∞β)	30	610-625
Allophycocyanin	(∞β)	104	650-660

Table III Purification and extraction recovery of PE and PC

Microorganisms	Extraction Method	Purification methods	Extraction Recovery	
			PE A563/A280	PC A615/A280
Nostoc Sps.strain HKAR-271	50mM potassium PO4 buffer + Sonication + repested freezing at pH 7.0	Sephaeryl Gel filteration chromatography + Dialysis + Precipitation (20 – 70% ammonium sulfate)	7.2	3.18
Nostoc Sps.strain HKAR-272	50mM potassium PO4 buffer + repeated freezing at pH7.0	Ammonium sulfate precipitation + Sepharyl Gel filteration chromatography +Hydrophobic interaction chromatography	97% recovery, 1.10 89% recovery, 6.37 83% recovery, 11.53	96% recovery, 0.92 80% recovery, 1.36 73% recovery, 5.75
Bangiaatropurpureα69	50mM potassium PO4 buffer + Sonication at 7.2	35% saturated ammonium sulfate + ialysis +Sephadex G-200+Reverse HPLC (RP-HPLC)	64.8% recovery, 2.47 91.3% recovery, 4.76 100% R 5.42	54.7% recovery, 0.77 68.3% recovery, 2.80 100% R 3.95
Porphyrayezoensis41	10mM potassium PO4 buffer + freezing thawing at pH6.8	Expanded bed chromatography (Phenyl sepharose) Anion exchange C (DEAE-Sepharose)	0.96 mg g-1 0.82 mg g-1	2.0 -2.5 4.5

REFERENCES

1. NamasivayamSenthilkumar, Veeraperumal Suresh, RamarThangam, ChidambaranKurijimalar, Ganapathy Kavitha, PitchaiMurugan, Soudarapandian Kannan and Ramasamy Rengasamy.Isolation and characterisation of macromolecular protein R-Phycoerythrin from Portieriahornemenii. Int. J. Biol. Macro mole. 2013;55:150-160.
2. Jothisaraswathi S., BabuB., Rengasamy R. J. Appl.Phycol. 2006; 18:161-166.
3. Saenger P., Hegerl E. J, Davie J. D and Work Group Mangrove. Ecosyst. IUCN Comm. Ecology, eds. 1983. Global status of mangrove ecosystems. Environmentalist 3(Suppl. 3).
4. Saravanan, K.R., IlangovanK. and Khan A. B.Floristic and macro faunal diversity of Pondicherry mangroves, South India. Trop. Ecol. 2008; 49: 91–94.
5. Balachandran N., Kichenamourthy S., Muthukumaran R., Jayakanthan M, Chandraseka S., PunethaA. and Sundar D. Diversity of true mangroves and their associates in the Pondicherry region of South India and development of a mangrove knowledge base. Journal of Ecology and the Natural Environment, 2009;1: 99–105.
6. Natalia Castejon, Maroussiaparailloux, AleksandreaIzdebska, RyszardLobinski, Susana CM, Fernandes. Valorization of the red algae Gelidiumsesquipedale by extracting a broad spectrum of minor compounds using green approaches. Marine drugs. 2021; 19:514.
7. Rengasamy K. R., Matomoodally M. F., Aumeeruddy M. Z., Zergin G., Xiao J., Kim D. H. Bioactive compound in seaweeds: An overview of their biological properties and safety. Food Chem. and Toxicol. 2020; 135: 110-113.
8. Ma R., Wang B., Chew E. T., Zhao X., Lu K., Ho Sh, Shi X.,Liu .L, Xic Y., Lu Y. et al. Comprehensive utilization of marine microalgae for enhanced co production of multiple compounds. Marine Drugs. 2020; 18:167-169.
9. Torres M. D., Florez Fernandez N., Dominguez H.. Integral utilization of red seaweed for bioactive production. Marine Drugs. 2019;17:314-317.
10. Alongi D. M., Mangrove forests: Resilience, protection from tsunamis, and responses to global climate change, Estuarine. Coastal and Shelf Sci., 2008;76: 1–13.
11. Van Bochove J., Sullivan E., Nakamura T., eds. 2014. The importance of mangroves to people: a call to action. Rep., U. N. Environ. Programme, World Monit. Cent., Cambridge, UK.
12. Feller I. C., Friess D. A., Krauss K. W., Lewis R. R. The state of the world's mangroves under climate change. Hydrobiologia. 2017; 803:1–12 17.
13. Herrero M., Sanchez Camargo A. D. P, Cifuentas A., Ibanez E. Plants, seaweeds, microalgae andfood by products as natural sources of functional ingredients obtained using pressurized liquid extraction and supercritical fluid extraction. TrAC Trends Anals. Chem. 2015; 71: 26-38.
14. Gallego R., Bueno M., Herrero M. Sub and super critical fluid extraction of bioactive compounds from plants, food by products, Seaweeds and microalgae – An update. TrAC Trends Anals.Chem.. 2019;116: 198-213.
15. Cardozo K. H. M., Guaratini T., Barros M. P, Falcon V. R., Tanon A. P,etal.,. Metabolites from algae with economical impact. Compar.Biochem. and Phy. 2007; 146: 60-78.
16. Milledge J. J., Commercial application of microalgae other than as biofuels and brief review. Reviews in Environ. and Biotech. 2011; 10:31-41.
17. Spolaore P, Joannis- Cassan C., Dusan E., Isambert A., Commercial applications of microalgae. J. Biosci.and Bioeng. 2006; 101:87-96.
18. Singh J., and Gu S., Commercialization potential of microalgae for biofuels production. Renew. and sustain.Ener. reviews. 2010;14:2596-2610.
19. Benomann J. R., Microalgae aquaculture feeds. J Appl.Phycol. 1992; 4: 233-245.
20. Brown M. R., Jeffrey S. W., VolkmanJ. K., Dunstan D. A., Nutritional properties of microalgae for mariculture. Aquacul. 1977; 151: 315-331.
21. Martin Akelsson, Francesco Gentiti. A single step method for rapid extraction of total lipids from green macroalgae. 2014; 9(2): 1-6.
22. Chisti Y., Biodiesel from microalgae. Biotech.Adv.. 2007;25: 294-306.
23. Khozin Goldberg, Iskandarov U., Cohen Z., LC-PUFA from photosynthetic microalgae – Occurrence, biosynthesis and prospects in biotechnology. Appl. Micro.and Biotech. 2011;91: 905-915.
24. Vitchez C., Forjan E., Cuaresma M., Bednar F, Garbayo L., et al. Marine carotenoids – Biological functions and commercial applications. Marine Drugs. 2011; 9:319-333.
25. Carrasco- Renado R., Escobar A., Carrera C., Guarrizo P, and Vallejo RA., Ferrandez- Aero FJ. Valorization of microalgae biomass as a potential sources of high value sugars and polyalcohols Lust. 2019;114.
26. Perez M. J., Felque E., Dominguez H., Antimicrobial action of compounds from marine seaweeds. Marine Drugs. 2016; 14, 52.
27. Grima F., Ullah Z., KaplanerE., Moujahad A., Eddoha R., Nasser B., Terzioglus P, Yilmaz M. A., ErtasA., Ozturk M., et al. *In Vitro* enzyme inhibitory properties, antioxidant activities and phytochemical fingerprints of five morocan seaweeds. South Afri. J. Bot.. 2020; 125: 152-160.
28. Grozdanic N., Stanojkovic T. P, Kljajiic Z., Etahiti S., Assobhei O., KenicRistic A., Srdic Rajic T., Kardum N., and Backoric S.,*In vitro* evaluation of antioxidant and antitumoral activities of marine algae *Gelidiumsesquipedals* and *Fucus spiralis*. Europ. J. Cancer. 2012; 48: 56-59.
29. Castejon N., Luna P, Senorans F. J., Alternative oil extraction methods from *Echium plantaginum*L. Seeds using advanced techniques and green solvents. Food Chem.. 2018; 144: 75-82.
30. Tatiana Perecirae,SoniaBarrosa, Swana Mendes, Renato A., Amaral, Juliana R. Dias, Teresa Beptista, Jorge A. Saraiva, Nuna M. Alves, and Marie M. Gil. Optimization of phycobiliprotein pigments extraction from red algae *Gracilariagracilis* for substitution of synthetic food colourants. Food Chem. 2020; 321: 1-9.
31. Soni B., Kalavadia B., Trivedi U., and Madamssar D., Extraction, purification and characterization of phycocyanin from *Oscillatoria quadripunctulata* isolated from the rocky shores of Bet Dwarka, Gujarat. India Pro.Biochem. 2006; 41(9): 2017-2023.
32. Somani R. R., Rastogi R. P, Patel R., and Mandamwar D., Recent advances in production, purification and applications of phycobiliproteins. World J.Biolog. Chem. 2016; 7(1): 100- 104.
33. Gabriela Kovaleski, Mariam Kholany, Lilia M. S., Dias, Sandra F. H., Correia, Rute A. S.,Ferreira,Jao A. P, Coutinho and Sonia P. M. Ventura. Extraction and purification of phycobiliproteins from algae and their applications. Front. in Chem. 2022; 1-19.
34. Mulders K. J. M., Lamero P. P., Martens D. E., and Wijiffels R. H., Phototrophic pigment production with microalgae biological constraints and opportunities. J.Phycol. 2022; 50:229-242.
35. Pagels F., Guedes A. C., Amaro H. M.,Kijjoa A., and Vasconcelos V., Phycobiliproteins from cyanobacteria chemistry and biotechnological applications. Biotech.Adv.. 2019; 37: 422-443.
36. Dumay J., Clement N., Morancaus M., and Fleurence J., Optimization of hydrolysis conditions of *Palmariapalmata*to enhance R-Phycoerythrin extraction. Biores. Tech. 2013; 131: 21-27.
37. Bryant D. A., Phycoerythrocyanin and phycoerythrin; properties and occurrence in cyanobacteria. J. Gen. Micro. 1992. 128; 835-844.
38. Dias L. M. S., Kovaleski G., Fu L., Dias T. R., Macario I. P. E., Correia S. F. H., et al. Uncovering the use of flucoxanthin and phycobilioroteins into solid matrics to its emission quantum yield and photostability. Appl. Sci.. 2022; 12: 5839-5911.
39. Munier J., et al. Physiochemical factors affecting the stability oftwo pigments: R-Phycoerythrin of *Grateloupiatururuturu* and B- Phycoerythrin of *Pophyridiumcruentum*. Food Chem. 2014; 150: 400-407.
40. Liu L. N., Chen X. L., Zhang X. Y., Xang Y. Z., and Zhou B. C., One step chromatography method for efficient separation and purification of R phycoerythrin from *Polyssiphoniaurcevlata*. J. Biotechnology. 2005; 116: 91100.
41. Lian Y. Z., Lin L. H., Yang Y. C., and Chao J. C. J., Gastroprotective effect of *Lyciumbarbarum* polysaccharides and C-phycocyanin in rats with ethanol-induced gastric ulcer. Int. J. Biol. and Macromole. 2020; 165: 1519-1528.
42. GallandIrmouli A. V., Pons L., Lucon M., Villaume C., Mrabat N. T., and

Guerant J. J., et al. One step purification of R-Phycoerythrin from the red macroalgae *Palmariapalmata* using Preparative polyacrylamide gel electrophoresis. J.chromato. Biomed. Sci. and Appl. 2000; 739: 117-123.
43. Sekar and Chandramohan. Phycobiliproteins as a commodity: Trends in applied research, patents and commercialization. J. Appl. and Phycol. 2007; 113-136.
44. Wang C., Zhoo Y., Wang L., Pan S., Liu Y., and Li S., et al. C-*Phycocyanin mitigates* cognitive impairment in doxorubicin-induced chemobrain: Impact on neuoinflammation, oxidative stress and brain mitochondrial and synaptic alterations. Neurochem. Res. 2020; 46: 149-158.
45. Kamble S. P., Vikhe G. P., and Chamle D. R.,Extraction and purification of phycoerythrin. A natural colouring agent from *Spirullina platensis*. J. Pharma. Chem. Biol. Sci. 2018; 6: 78-84.
46. Pan Q., Chen M., Li J., Wu Y., Zhen C., and Liang B.,Anti tumor function and mechanism of phycoerythrin from *Porphyrahaitanensis*. Biol. Res. 2013; 46: 87-95.
47. Jung S. M., Park J. S., Shim H. J., Kevan Y. S., Kim H. G., and Shin H. S., Antioxidative effect of phycoerythrin derived from *Grateloupiafilcina* on rat primary astrocytes. Biotech.Biopro. Eng. 2016; 21: 676-682.
48. Tan H., Gao S. T., Zhuang Y., Dong Y., Guan W., and Zhang K., et al. R-Phycoerythrin induces SGC-7901 apotosis by arresting cell cycle at S phase. Marine Drugs. 2016; 14: 166-169.
49. Beattle S. W., Morancais M., Deleris P., Flusence J., and Dumany J., Extraction of phycocyanin and phycoerythrin pigments. In B Charrier T, WichandandCRK Reddy (Eds.) Protocols of macroalgae Research. CRC Press. 250-266.
50. Mittal R., Tavanandi H. A., Mantri V. A., and Raghuvaran K. S. M., Ultrasound assisted methods for enhanced extraction of phycobilliprotein from marine macro algae. *Gelidiumpusilium*(Rhodophyta). Ultrasonics Sonochem. 2017; 38:92-103.
51. Subhakar M. P., Jagatheesan A., Perumal K., and Arunkumar K., Methods of phycobiliprotein extraction from Gracilariacrassia and its applications in food colourants. Algal Res. 2015; 8: 115-120.
52. Martins M., and Ventura S. P. M., Emerging seaweed extraction techniques using ionic liquids in editors MD Torres, S Kraan and Deminguez Sustain, Seaweed Technology Amsterdam, Netherlands, Elseveir. 2020. 287-311.
53. Martinez J. M., Delso C., Alvarez L., and Raso J., Pulsed electric field permeabilization and extraction of phycoerythrin from *Porphyridiumcruentum*. Algal Res. 2019; 37: 51-56.
54. Pereira T., Barrosn S., Mendes S., Amaral R. A., Dias J. R., and Baptista T., et al. Optimization of phycobiliprotein pigments extraction from red algae *G.gracilis* for substitution of synthetic food colourant. Food Chem. 2020; 321: 126-128.
55. Mittal R., Sharma R., and Raghavasan K.,Aquous two phase extraction of R-Phycoerythrin from marine macro algae, *Gelidiumpusilum*. Biores. Tech. 2019; 280: 277-286.
56. Francavilla M., Manara P., Kamalirou P., Monteleone M., and Zabaniotou A., Cascade approach of red macroalgae *Gracilariagracilis* sustainable valorization by extraction of phycobiliproteins and pyrolysism of residue. Biores. Tech. 2015: 184: 305-313.
57. Ibanez-Gonzilez M. J., MazzucaSobczuk T., Redondo Mirando R. M., Molina Grima E., and Cooney C. I.., A novel vortex flow reactor for the purification of Phycoerythrin from *Porphyridiumcruentum*. Algal Res. 2016; 37: 51-56.
58. Dumay J., Clement N., Morancais M., and Fleurence J., Optimization of hydrolysis conditions of *Palmaria palmate* to enhance R-Phycoerythrin extraction. Bioresource Technology. 2013; 131: 21-27.
59. Ngevyen H. P. T., Morancaus M., Fleurence J., and Dumay J.,*Mastocarpusstellatus* as a source of R-Phycoerythrin optimization of enzyme assisted extraction using response surface methodology. J. Appl. Phycol. 2016; 29: 1563-1570.
60. Choi W., and Lee H., Effect of ultrasonic extraction on production and structural changes of C-phycocyanin from marine *Spirulina maxima*. Int. J. Mol. Sci. 2018; 19: 220-311.
61. Rodrigues R. D. P., Castro F. C., Santiago Ageciar R. S., and Rocha M. V. P., Ultrasound assisted extraction of phycobiliprotein from *Spirulina (Arthrospira) platensis* using probic ionic liquids as solvent. Algal Research. 2018. 31; 454-462.
62. Rodrigues RDp, Lima PF, Santiago Aguiaz Rs and Rocha MVP. Evaluation of protic ionic liquids as potential solvents for the heating extraction of phycobiliproteins from *Spirulina (Arthrospira) platensi*. Algal Res. 2019; 38: 101391.
63. Juen C., Cherouvrier J. R., Thiery V., Gagez A. L., Berard J. B., andJaguet N., et al. Microwave assisted extraction of phycobiliprotein from *Porphyridiumpurpurem*. Appl. Biochem. and Biotech. 2014; 175: 1-5.
64. Rito-Palomares M., Nunez L., and Amador D., Practical application of aqueous 2 phase systems for the development of a prototype process for C-phycocyanin recovery from *Spirulina maxima*. J. Chem. Tech. and Biotech. 2001; 76: 1273-1280.
65. Lee Z., Nishizawa M., Shimizu Y., and Saeki H.,Anti inflammatory effects of dulse (*Palmariapalmata)* resulting from silultaneous water extraction of phycobiliprotein and chlorophylla. Food Res. 2017; 100: 514-521.
66. Gargouch N., Karkouch I., Elleuch J., and Elkahouri S., et al. Enhanced B-Phycoerthrin production by the red microalgae *Porphyridiummarinum*. A powerful agent in industrial applications. Ind. J. Biol.Maromol. 2021; 120: 297313.
67. Senthilkumar N., Suresh V., and Thangam et al., Isolation and characterization of macromolecular protein phycoprotein from *Portieriahornemannii*. Int. J. Biol. and Macromole. 2013; 55:150-160.
68. Munier M., Morancais M., Deleris P., and Flourence J., et al. Purification of R Phycoerythrin from a marine macroalgae *Gracilariagracilis* by anion exchange chromatography. J. Appl. Phycol. 2019; 32: 553-559.
69. Tripathi S. N., Kapoor S., and Shrivastava A,. Extraction and purification of an unusual phycoerythrin in a terrestialdesication tolerant cyanobacterium *Lynghyaacboricola*. J. Appl.Phycol. 2007; 19: 441-447.
70. Kim J. J., Jeon Y. N., Noh J. H., and Lee M. Y., Isolation and characterization of a new phycoerythrin from the cyanobacterium *SynechococcusSps*. ECS-18. J. Appl. Phycol. 2010; 23: 137-142.
71. Zhao H. G., Refractive index dependent optical property of carbon dots integrated luminescent solar concentrators. J. Lumin. 2019; 211: 150-156.
72. Rossano R., Ungaro N. D., Ambrosio A., Liuzzi G., and Riccio P., Extracting and purifying R Phycoreythrin from Mediterranean red algae *Corallina elongataellis* and *Solander*Sps. J. Biotech. 2003; 101: 289-293.
73. Cai C., Wang Y., Li C., Guo Z., and Jia R., Purification and phtodynamic bioactivity of phycoerythrin and phycocyanin from *Porphyrayezoensisveda*. J. Ocean Univ. China. 2014; 13: 479 – 484.
74. Kamaujiya V. K., and Sinha R. P., An efficient method for the separation and purification of phycobiliproteins from a rice field cyanobacterium *Nostoc*Sps Strain HKAR-11. Chromatographia. 2016b; 79: 335-345.
75. Kamaujiya V. K., and Sinha R. P.,Thermkineticstabilty of phycocyanin and phycoerythrin in food grade perservatives. J. Appl.Phycol. 2016a; 28: 10631070.
76. Punampalam R., Khon K. S., and Sit N. W.. Evaluation of antioxidant antpropreties of phycobiliprotein and phenolic compounds extracted from *Bangiaatropurpurea*. Mal. J. Fund. Appl. Sci. 2018; 14; 289-297.
77. Niu J. F., Chen Z. F., Wang G. C., and Cheng Z., Purification of phycoerythrin *PorphyrayezoensisUeda*. J. Ocean Univ. China. 2010; 13: 578 – 584.
78. Guedes A. C., Amaro H. M., and Malcata F. X., Microalgae as source of carotenoids. Marine Drugs. 2011; 9: 625 – 644.
79. Manivasagan P., Bharathiraja S., Moorthy S. M., Mondal S., and Sen H. et al., Marine natural pigments as potential sources for therapeutic applications. Crtical Rev. Biotech. 2011; 38: 745-761.
80. Yang M., Cui M., Wang Y., Li T., and Song C., et al., Sensitive and selective detection of Cu2+ ions based on fluorescent Ag nanoparticles synthesized by R Phycoerythrin from marine algae*Porphyrayezoensi*.Ecotoxicol. and Environ. 2019; 168: 356-362.
81. Sun Y., Zang L., Lau C., Zhang X., and Lu J., Sensitive detection of transcription factor by coupled fluorescence encoded microsphere with exonuclease protection. Talanta. 2011; 229: 1232-1272.
82. Ghosh and Mishra S., A natural cyanobacterial protein C- Phycoerythrin as an HS- selective optical probe in aqueous systems. Spectro. Acta Part A. Mol. Biol. Spectro.2020;239: 118467.
83. Ghosh T., Chatterjee S., Bhayani K., and Mishra S.,Natural cyanobacterial protein C- Phycoerythrin as an Hg 2+ selective fluorescent probe in aqueous systems. New J. Chem. 2020; 44: 6601-6609.
84. Xu Y., Hou Y., Wang Y., Li T., and Song C., et al.,Sensitive and selective detection of CU2+ ions based on fluorescent Ag nanoparticles synthesized by R-Phycoreythrin from marine algae *Porphyrayezensis*. Ecotox. and Environ. Safety. 2019; 168: 356-362.
85. Loney A. C., Tschanz A., and Heck A. J. R., Connecting colour with assembly in the flurescent B-Phycoerythrin protein complex. FEBS. 2017; 285: 178-187.
86. Kaldmae M., Sahin C., Saluri M., Marklund E. G., and Landoeh M., A strategy for the identification of protein architecture directly from ions mobility mass spectrometry data reveals stabilizing sub unit interactions in light harvesting complexes. Prot. Sci.2019; 28: 1024-1030.
87. Correio S. F., Bastos A. R. N., Martins M., Macarrio I. P. E., and Velso T., et al., Bio solar energy harvesting for onsite mobile optical temperature sensing in smart cities. Adv. Sci. 2022; 9: 2104801- 2104810.
88. Osman A., Salama A., Mahmoud K. E., and Sitoby M., Alleviation of carbon tetrachloride induced mitochondrial dysfunction and oxidative stress. Mol. Cell and Biochem. 2020; 406: 183-197.
89. Yeshimoto S., Okado K., and Hayashi O.,Immunoregularty and anti inflammatory actions of phycocyanoerythrin on CaCo2/ U937 cells co culture as a model of the intertestinal barrier. Func. Food Heal.2019; 9: 466-483.
90. Li W., Su H. N., Pu Y., and Chen J., P B P molecular structure, production, applications and prospects. Biotech. Adv. 2019; 37: 340-353.
91. Chaubey M. G., Patel S. N. K., Rastogi R. P., and Srivastava P., et al. Therapeutic potential of cyanobacterial pigment protein phycoerythrin. *In Silico* and *In vitro* study of BACE. Interaction and *In vitro*Sps. reduction. Int. J. Bio. and Macromol. 2019; 134: 368-378.
92. GallandIrmouli A. V., Pona L., Laqon M., and Villacne C., et al., One step purification of R-Phycoerythrin from the red macroalgae *Palmariapalmata* using preparative polyacrylamine gel electrophoresis. J. Chromat.Biomol. Sci. Appl. 2000; 739: 117-123.
93. Manirafasha E., Nichikubwimma T., Zang X., Lu Y., and Jung K.,Pycobilliproteins potential microalgae derived pharmaceutical and biological reagent. Biochem. Eng. J. 2016; 109: 282-296.
94. Mishra S. K., Shrivastav A., Panchan L., Jain D., and Mishra S., Effect f preservatives for food grade C- Phycobilliерythrin isolated from marine cyanobacteria *Pseudanabaena*Sps. Int. J.Biol.andMacromol. 2010; 47:997602.
95. Kannajiya and Sinha. Thermokinetic stability of phycocyanin and phycoerythrin in food grade perservatives. J. Appl.Phycol. 2016; 28: 10631070.
96. Galatovic R., Martiner M., Gifuentes A., Ibaner E., and Herrero H., Development of a green downstream process for valorization of *Porphysidiumcruentum* biomass. Mole. 2019; 24: 1564.