Abstract

The study depicts the use of various green extraction techniques including supercritical fluid extraction, ultrasound assisted extraction, enzyme assisted extraction and subcritical water extraction to utilize each fraction of an algal biomass for its conversion to value added products. In our research, we used the pilot scale algal biofilm reactor (100 L) to cultivate the microalgal consortia PA6 (*Chlorella* sp. and *Phormidium* sp.), and we procured red, green, and brown macroalgal biomass from the coasts of Tamil Nadu and Chilika lake, Orissa, India. The physicochemical characterization of biomass revealed that it contains high amount of carbohydrate (~ 20.4 ± 0.5 %), protein (~ 30 ± 0.9 %), fiber, dietary fiber, and minerals. The first step towards the valorization of algal biomass was extracting lipids using supercritical fluid extraction technique (SFE). The maximum lipid yield (~ 3 ± 0.4 %) was obtained by PA6 biomass which was found rich in polyunsaturated fatty acids content (20.68 %) with the presence of eicosapentaenoic acid, docosahexaenoic acid, α-linolenic acid, linoleic acid and arachidonic acid. The residual biomass after SFE showed recovery of 27.1 ± 0.9 mg g^{-1} of total carotenoids, which could have applications in food industry.

The second stage of biomass valorization focuses on the polysaccharides contained in the SFE residual biomass. Using subcritical water (ScW) approach, ~ 14 % fucoidan and ~ 48 % of sodium alginate was recovered from SFE residual brown macroalgae. Furthermore, ultrasonication assisted approach recovered ~ 56 % of κ-carrageenan from SFE residual red macroalgae. Fucoidan and κ-carrageenan had sulfate content of 25 % and 28.5 %, respectively, which contributes to their biological activity. Analysis of monosaccharide composition of fucoidan reveals that ScW approach recovers highest content fucose (54.37 %) and galactose (83.32 %) with small amounts of arabinose, glucose, glucuronic acid, mannitol, mannose, rhamnose and xylose. The alginate derived from ScW revealed the presence of mannuronic (M) and guluronic acids (G) with M/G ratio greater than 1, fulfilling

WHO and FAO guidelines for the nutraceutical and pharmaceutical industries. κ-carrageenan exhibited a monosaccharide content of 3,6-anhydrogalactose (38.7 %) and galactose (23.1 %), as well as rheological properties within FAO limitations that can be explored for food-grade applications. In addition, it was shown that these extraction methods are selective for polyphenolic and flavonoid compounds, hence enhancing their antioxidant potential. These polysaccharides exhibited strong *invitro* 2,2-diphenyl-1-picrylhydrazyl scavenging activity (up to 88 %), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid radical scavenging activity (up to 80 %) and ferric reducing antioxidant power value. Hence, polysaccharides from macroalgae were recovered using environmentally friendly, sustainable methods with potential uses in the food, pharmaceutical, and cosmetics industries.

The final stage of biomass valorisation concentrates on the proteins present in the SFE residual biomass with the goal of producing a minimal quantity of waste by products. The protein yield in algal biomass using different pre-treatments including ultrasonication and homogenization varied from 0.1 mg g^{-1} to 55.3 mg g^{-1}. *P. tetrastromatica* exhibited the maximum protein yield, therefore it was further optimised using ScW hydrolysis under various temperature and time conditions. The optimum conditions for obtaining the maximum protein (127.2 ± 1.1 mg g^{-1}), free amino acids (58.4 ± 1.0 mg g^{-1}), highest degree of hydrolysis (58.8 ± 1.2 %) and low molecular weight peptides (< 650 Da) were found to be 220 ˚C for 10 minutes. The amino acid profiling of the hydrolysate revealed that it contains 45 % essential amino acids, with the highest concentration of methionine (0.18 %), isoleucine (0.12 %) and leucine (0.10 %). It was found that the hydrolysate contains phenolics (23.9 ± 1.4 mg GAE g^{-1}) and flavonoids (1.23 ± 0.1 mg QE g^{-1}), which are largely responsible for antioxidant activity. The hydrolysate effectively inhibits acetylcholinesterase (up to 53 %) and α-amylase (up to 16 %) *invitro*, which can help in prevention of Alzheimer's disease and diabetes mellitus. Consequently, this study reveals that utilising eco-friendly subcritical water hydrolysis method, ~ 79 % of protein was recovered from *P. tetrastromatica*, which might be an effective source as bioactive peptides in various nutraceutical, pharmaceutical and cosmeceutical applications.

In addition, the sulfated polysaccharides carrageenan and fucoidan extracted from red and brown macroalgae utilizing green emerging techniques were evaluated for their potential in inhibiting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by targeting its main protease ($3CL^{pro}$) and receptor binding domain (RBD) through *invitro* and *insilico* approach. According to the findings of our *invitro* study, κ-carrageenan from red macroalgae was effective in inhibiting the $3CL^{pro}$ of SARS-CoV-2 by up to 93 %, whereas fucoidan from brown macroalgae was effective in inhibiting the $3CL^{pro}$ of SARS-CoV-2 by up to 97 %. However, none of the sulfated polysaccharides were found to be active against the RBD protein. Molecular docking investigations of fucoidan revealed the lowest binding energy (-6.0 kcal mol^{-1}) for $3CL^{pro}$ compared to carrageenan (-5.2 kcal mol^{-1}). Molecular dynamics simulations results revealed that carrageenan and fucoidan successfully binds to the active site of the $3CL^{pro}$ while retaining the structural integrity and stability of protein-ligand complexes. The Absorption, Distribution, Metabolism, and Excretion (ADME) properties have been met by both compounds, although only fucoidan obeyed Lipinski's rule of five. The toxicity parameters suggested that neither of the compounds exhibit AMES toxicity, hepatoxicity and skin sensitivity. Hence, carrageenan and fucoidan from macroalgae could act as possible inhibitors in regulating the function of the $3CL^{pro}$ protein, hence inhibiting viral replication and being effective against COVID 19.

Hence, the study brings out a promising pathway to valorize algal biomass by extracting multiple value-added compounds using green techniques for multiple industrial applications, thereby targeting a minimum waste generation approach. Besides, this study also highlights the limitations for practical implications at industrial scale. For this, a systematic study on industrial scale cultivation and harvesting of algal biomass along with its life cycle assessment and techno-economic analysis is recommended to ensure recovery of similar types of industrially relevant food/pharma value products in a sustainable manner.