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Pharmacological Importance of *Gracilariopsis Lemaniformis* Seaweed

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Abstract

Ocean covers about one-third of the earth and is considered as a vital natural resource. Seaweeds accompany a large areas of the ocean coastal area, which are considered as among the primary producers. Many seaweeds have food values and so many indigenous populations relay on them as their nutrient source. Besides, seaweeds are also rich in many bioactive compounds having pharmacological significance. Seaweeds contain higher number of proteins, vitamins, minerals, essential fatty acids, polysaccharides and so on. Gracilariopsis lemaniformis is a red sea alga and is also rich in bioactive compounds having medicinal values. In this review article, we focused on the pharmacological importance of this species based on recent updates of literature survey and we discussed here about its antimicrobial, antitumor, antidiabetic and antioxidant properties, which would demand its therapeutic relevance for future drug development.

Keywords: *Gracilariopsis lemaniformis*; Pharmacology; Antimicrobial; Antitumor; Antidiabetic; Antioxidant

Introduction

Now a day, the concept of blue economy emerges all over the world. As population is increasing, so that to meet the at least the food protein and medicinal demand, peoples are looking for an alternative food source rather terrestrial for a sustainable development. People have made a living from the sea in various ways since ancient times, but the notion of blue economy emerged under unexpected circumstances, because of traditional infrastructure development, this treasured environment has progressively been disrupted by immense demand on limited resources. Because the world is losing its equilibrium because of industrialization, urbanization, and fast population expansion, people have been looking for new methods to make a living. As a result, individuals are taking various actions to improve their economic standing as well as their infrastructure. because of this evolution Along with previous notions of development, the United Nations Conference on Sustainable Development (took place in Rio de Janerio, 2012) has credited as being the new concept, 'Ocean Economy' or 'Blue Economy' [1]. Oceans take up roughly 71 percent of the surface of the globe and are home to approximately 95% of the biosphere's total mass. This is a vast source of wealth. The activities of ocean economy include generation of new resources, extraction of non-living resources, construction of the built environments, commerce, tourism, ocean observation and forecasting, indirect contributions to environments such as carbon sequestration, coastal protection etc. with an indicative annual gross revenue of $2,626 billion [2].

The Bay of Bengal, a part of Indian Ocean, is known as the largest triangular basin in the world. It is the source of 64 larger marine ecosystems in the world. Total area of Bay of Bengal is 2,172,000 square kilometers. The Bay of Bengal lies roughly between latitudes 5o and 22oN and longitude 80o and 90oE. it is verged by Myanmar and Malay Peninsula to the east, Sri Lanka and India to the west and Bangladesh to the north. Bangladesh achieved a tremendous success by the maritime border settlement with Myanmar and India which cover about 121,110 sq.km. of sea area Exclusive Economic Zone (EEZ) in Bay of Bengal which was about 1,18,813 sq.km. Bangladesh is a nation with a high population density but also has a low income per capita, a poor level of food security, and a significant issue with malnutrition [3]. Proper utilization and sustainable management of marine resources can promote Bangladesh into a middle-income country [4].

Ocean is a huge reservoir of diversified organisms with beneficial potentials to human. The diversified characters from primary producers to tertiary levels are due to the harsh environment in ocean to survive leads them to generate many secondary metabolites, which possesses pharmaceutical, nutraceutical and food values [5-7]. Marine algae or seaweeds are the vital components of the marine ecosystem possessing pharmacological reservoir [8-10].

Algae, singular alga, are members of the kingdom Protista, which are mostly aquatic photosynthetic creatures. Algae come in a variety of sizes, from microscopic Micromonas species to huge kelps that may exceed 60 meters (200 feet) in length. Their photosynthetic pigments are more diverse than plants', and their cells contain characteristics not seen in either plants or animals. Algae are commercially essential as a source of crude oil, food, and a variety of medicinal and industrial goods for humans, in addition to their ecological responsibilities as oxygen providers and the nutritional foundation for practically all aquatic life. Some algae produce lots of bioactive compounds due to sustain in the harsh environment that are of great pharmacological values [5]. Besides, from long since algae or seaweeds are being used in the indigenous population as food and for medication. Now a day, seaweeds are used as cosmetic materials, food industry, pharmaceutical industry, and medical purposes due to high content of essential fatty acids, vitamin, minerals, proteins, and polysaccharides [11,12].

Algae or Seaweed has been used as a food source since pre-historic times. More than 2000 years ago, extracts of marine creatures were utilized as medicine, according to historical sources. Many seaweed species have been utilized as herbal medicine in China [13,14]. The usage of numerous seaweed species is described in the Oriental medical treatise Dong Ui Bo Gam, published in 1613, for the treatment of fever, tumors, and swelling [15]. The use of marine algae for food and research has exploded in recent decades, resulting in a multibillion-dollar industry. Seaweed-based items have found their way into cuisines, cosmetics, medications, and nutritional supplements. Biological activity of possible medicinal significance has been documented in several seaweeds and their extracts, including anti-inflammatory, cytotoxic, and antibacterial actions. Many studies have focused on marine microorganisms and seaweeds in the search for new drugs made from natural products [16-18]. In Marine, there are around 50 lakhs (50,000,00) species that are practically unexplored sources of secondary metabolites. Apart from their potential ecological/industrial significances such as controlling reproduction, settlement/biofouling, and feeding deterrents, those compounds already isolated from seaweeds are providing valuable ideas for the development of new drugs against cancer, microbial infections, and inflammation [19-22].

Based on the kind of colors, morphological, anatomical, and reproductive features, seaweeds, or marine algae are divided into four groups: *Chlorophyceae* (green algae), *Phaeophyceae* (brown algae), *Rhodophyceae* (red algae), and *Cyanophyceae* (blue-green algae) [23]. Seaweed comes in a variety of forms, colors, and sizes and may be found in all of the world's oceans. They're particularly common in rocky coastal locations with shallow water, especially when they're exposed at low tide. Seaweeds have been harvested and consumed by coastal people all over the world since the dawn of time. Seaweeds contribute to the ocean's primary production, making seaweed beds a highly productive and dynamic ecosystem.

Natural abundances of seaweeds have been observed in Bangladesh, particularly in the south- eastern part of the peninsula and offshore islands, such as Saint Martin Island, which has a rocky substratum that is suitable to natural seaweed development. The littoral and sub-littoral zones of St. Martin's Island are home to naturally growing seaweeds. As population is increasing day by day throughout the world and land is not increasing, so that to compensate the need of food as well as medicinal drug support, scientists are looking for alternative sustainable source. As ocean covers around 71% surface of the globe, so that seaweed from the coastal region would be a rich and alternative source of pharmaceutical agent as well as food supplements. The *Gracilariopsis lemaniformis*, red sea algae, usually grow in the coastal region of the sea and avails food values and pharmacological agents. Based on the demand of blue economy, an attempt has been taken in this study to compile the more recent pharmacological values of Gracilariopsis lemaniformis on the basis of scientific literatures.

Seaweeds in Bangladesh

Even though Bangladesh has 193 seaweed species belonging to 94 genera, including 19 commercially significant ones, they are mostly untapped, except by Mog and Rakhyine tribal populations and seaweed collectors [24]. Between October and April, 60 seaweed species may be found in the Sundarbans, 155 species in Cox's Bazar, and 140 species in St. Martin's Island, although they are most prevalent in January and March [24]. According to a survey of sub-littoral seaweeds on St. Martin's Island, there are at least 37 seaweed taxa on the island, with 11 belonging to the Chlorophyceae, 14 to the Phaeophyceae, and 12 to the Rhodophyceae, and their distribution, abundance, and diversity varying according to temperature, location, and other factors [25]. The samples for this study were gathered from January to June of 2007, despite the fact that it was published in 2018. A further investigation in April 2013, which was published in 2015, discovered two additional *Phaeophyceae* taxa [26]. Bangladesh is home to 197 seaweed species (95 red algal species, 46 green algal species, and 56 brown algal species) [26]. As a result, there appears to be a lack of scientific consensus about the quantity of seaweeds in Bangladesh. Hopefully, the National Seaweed Database of Bangladesh (https://nib.portal.gov.bd/site/page/ad05f4de-1a36-4302-aed0-591cbd43322a/National Seaweed- Database) Bangladesh's National Institute of Biotechnology (NIB) is now employing DNA barcoding to identify seaweed species. Bangladesh may earn a lot of money by cultivating and exporting edible seaweeds [27]. Furthermore, one of the advantages of integrated aquafarming is

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the possibility of producing value-added goods such as biofuels from seaweeds [28]. The poor coastal communities of Bangladesh may be able to supplement their income by farming seaweeds, however there is currently no viable large-scale seaweed mariculture system in place, and local residents have no clue when such an enterprise would be possible [29]. Biological elements, environmental factors, technological and scientific knowledge, and socio-economic factors (political and legal system, economic demand, and production cost) may all impact the fate of seaweed agriculture [30]. Some of the key problems of such initiatives in Bangladesh are a lack of scientific understanding, suitable technology, and experienced staff [30]. Fortunately, several studies are already discovering places in Bangladeshi coastal areas that are appropriate for seaweed production. The possibility of seaweed culture, particularly the growth of red seaweed *Hypnea spp.*, is being researched at St. Martin Island and the Bakkhali and Inani coasts of Cox's Bazar [31-33].

Bioactive Compounds of Seaweeds and their Effects

Bioactive substances derived from seaweeds, in addition to entire seaweeds and seaweed-based food items, provide several health advantages [34]. Because of the hypervariable character of the marine environment [35], seaweeds have a large number of secondary metabolites that might be useful in the medical and pharmaceutical sectors [5,12]. It's worth noting that bioactive substances can come from both primary and secondary metabolites [36]. Seaweeds have been used to extract bioactive peptides, polysaccharides (laminarans, fucans, galactans, ulvan, alginates, carrageenan, etc.), polyphenols, fatty acids (omega-3 fatty acids such as docosahexaenoic acid, eicosapentaenoic acid, etc.), vitamins (both fat soluble vitamins such as vitamin A, vitamin D, vitamin E [12,34]. Antimicrobial, anticancer, antioxidant, antiplasmid, cytotoxic, anticoagulant, neuroprotective, cardioprotective, antihypertensive, wound healing, antiobeseogenic, hypocholesterolemic, antidiabetic, and many more actions are among the properties of these substances [34,37-39]. Many of these chemicals are only found in seaweeds, and they may have more bioactivity than those found in terrestrial plants [40,41]. A database called Seaweed Metabolite Database (SWMD) was built to keep focused on seaweed-derived bioactive chemicals that target the pharmaceutical sector [42]. Seaweeds have been utilized in traditional herbal therapy for thousands of years [43,44]. Furthermore, they are now being researched as possible prebiotic sources [45]. Sodium oligomannate (GV-971), a seaweed-based medicine newly authorized in China for the treatment of Alzheimer's disease, operates by altering the gut flora [46].

Gracilariopsis Lemaneiformis Seaweed

Classification and distribution

Kingdom: *Plantea*, Sub-kingdom: *Biliphyta*

Phylum: *Rhodophyta*, Sub-phylum: Eurhodophytina

Class: *Florideophyceae*, Sub-class: *Rhodymeniophycidae*

Order: *Gracilariales*

Family: *Gracilariaceae*

Subfamily: *Gracilarioideae*

Genus: *Gracilariopsis*

Species: *Gracilariopsis lemaneiformis*

Gracilaria lemaneiformis, commonly known as asparagus, sea hair vegetable, thread vegetable, and gracilaria, is a kind of economic red algae that belongs to the Rhodophyta Phylum, Gigartinales Order, *Gracilariaceae* Family, and *Gracilaria* Genus, and has a long culinary history (Figure 1) [47,48]. More than 6500 species of red algae or Rhodophyta make up an old and distinct group of photosynthetic eukaryotes. *Gracilaria lemaneiformis (G. lemaneiformis)* [49], a red alga formerly known as *Gracilaria lemaneiformis (G. lemaneiformis)*, was formerly considered to be found in China, Japan, Peru, and North America. *Gp. lemaneiformis*, on the other hand, was not found globally, according to Gurgel et al. [50], and was only found in the Peruvian region. *Gp. lemaneiformis*, behind Saccharina and Pyropia, is China's third-largest farmed seaweed, with an annual dry weight of 246 million kg. *Gp. lemaneiformis* wild populations are mostly found along China's northern coast, although the high-temperature-tolerant cultivar 981 may be grown anywhere from the north to the south [51].

With mild warming, certain species, such as *Gracilariopsis lemaneiformis*, may benefit from increased photosynthesis while respiration stays essentially unaltered [52]. Other marine organisms may benefit from the nutrients provided by *Gracilariopsis lemaneiformis*. It contributes to the ecology as a primary producer. It may trap watery detritus in the mangrove ecosystem, which can be eaten by crustaceans like *Paranebalia belizensis* [53]. *Gracilariopsis lemaneiformis* exudates are high in fractional dissolved combined neutral sugars (DCNSs), which can enhance bacterioplankton development [54]. The biochemical makeup of *Gracilariopsis lemaneiformis* varies according to the season. For example, the lipid and total energy contents of *Gracilariopsis lemaneiformis* collected in the winter may be much greater than those of the same species obtained in the summer from the same location [55]. Variations in bioactivities of *Gracilariopsis lemaneiformis* may be caused by seasonal changes in composition [56]. Chemical compositions and bioactivities of *Gracilariopsis lemaneiformis* have been reported to vary spatially [56,57]. Available nutrients [58], ocean acidification events [59], and other factors can influence *Gracilariopsis lemaneiformis* productivity.

G. lemaneiformis has been developed for use as a delectable human food source, abalone feed, and agar production. Furthermore, *G. lemaneiformis* is capable of absorbing nitrogen and phosphate from saltwater as well as preventing the growth of red tide-causing microalgae [60]. *Gracilariopsis lemaneiformis* has

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been widely farmed in recent years in coastal locations such as Japan, Korea, Russia, and various Chinese provinces (Shandong, Liaoning, Guangdong, Heilongjiang, Jilin, Fujian, and other coastal cities). Dry Gracilariopsis output in China has already reached 368,967 tons, with a cultivated area of 10,459 ha in 2020, making it the second biggest farmed seaweed in China [61]. *Gracilariopsis lemaneiformis* can be consumed raw or processed into feed, food additives, moisturizers, gels, medications, and other products. It is widely used in feed, food, cosmetics, medicine, the chemical industry, and other fields [62]. *Gracilariopsis lemaneiformis* is commonly used to make agar, with 91 percent of agar produced in 2015 coming from Gracilaria algae like *Gracilariopsis lemaneiformis* [63]. *Gracilariopsis lemaneiformis* may not only provide economic advantages by being processed into various goods, but it can also provide ecological benefits by balancing nitrogen and phosphorus levels, reducing heavy metal pollution, improving the water environment, and repairing the ecosystem [48].

Figure 1: (A) Morphology of *Gracilariopsis lemaneiformis*, (B) Structure of *G. lemaneiformis*. (C) *G. lemaneiformis* collected from Bay of Bengal.

Pharmacological prospects

Gracilariopsis lemaniformis possesses lots of pharmacological properties such as antimicrobial, cytotoxic, antitumor, and so on. Here few of the therapeutic properties along with bioactive compounds found in this species are discussed.

Antioxidant properties: Cells produce lots of free radicals or reactive persulfides and/or reactive oxygen species (ROS) during cellular metabolism [64]. The reactive oxygen species are harmful to cells and antioxidant system in the body usually scavenges these free radicals to maintain homeostasis [65]. Several reports suggests that seaweeds are rich source of antioxidants and Gracilariopsis lemaniformis belongs to one of those seaweeds. In a very recent study Hu et al. [66] reported that bioactive peptides from G. lemaniformis generated by alkaline protease showed strong antioxidant properties. Three bioactive peptides PGPTY, LSPGEL, and VYFDR are strong antioxidants and among them PGPTY showed strong Keap1 docking, negative regulator of master gene regulator Nrf2 for antioxidant response pathway [67,68], revealed antioxidant potential of this seaweed. Upon exposure to H_2O_2 in HepG2

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cell, these antioxidant peptides also showed increased level of SOD activity leading to extended viability of cells by scavenging ROS [66], which suggested that bioactive peptides obtained from *G. lemaniformis* would be a rich source of antioxidants. Another group [69] also showed that bioactive peptide Glu-Leu-Trp-Lys-Thr-Phe generated by protease hydrolysis (trypsin, pepsin, papain, alfa-chymotrypsin, alcalase) also demonstrated significant DPPH scavenging activity. Low molecular weight polysaccharides from *G. lemaniformis* also possess strong antioxidant properties. Fang et al. [70] and the group reported that utilizing the enzymes pectinase, glucoamylase, cellulase, xylanase, and dextranase, *G. lemaniformis* polysaccharides (GLP) were converted into low-molecular-weight polysaccharides, namely GPP, GGP, GCP, GXP, and GDP, and their antioxidant properties were examined in vitro and in human fetal lung fibroblast 1 (HFL1) cells. GDP demonstrated the strongest antioxidant activity, while degraded GLP showed stronger antioxidant activities than native GLP. Four polysaccharide fractions (GDP1, GDP2, GDP3, and GDP4) with high antioxidant abilities (hydroxyl radical scavenging activity, DPPH radical scavenging activity, reduction capacity, and total antioxidant capacity) were obtained after the optimization of degradation conditions through single-factor and orthogonal optimization experiments. In human fetal lung fibroblast 1 (HFL1) cells, increased cytoprotective activities were observed when cells were pretreated with GDP and it dramatically increased cell viability, lowered the levels of reactive oxygen species and malonaldehyde, boosted antioxidant enzyme activity, increased mitochondrial membrane potential, and reduced oxidative damage under H_2O_2-induced oxidative damage [70]. Another group also suggested that short chain polysaccharide GCP provided extensive protection to splenocyte damaged by H_2O_2 [71].

Neoagarooligosaccharides (NAOs) generated from *G. lemaniformis* also possess antioxidant activities. It was reported that NAOs can be produced from this species and *G. lemaniformis*-derived NAOs had strong antioxidant activities that was proved through DPPH, ABTS and free radical scavenging experiments [72]. Transcriptome sequencing of *G. lemaniformis* also revealed that upregulation of antioxidant genes [73]. Polysaccharide derived sulfated and acetylated derivatives from *G. lemaniformis* are strong antioxidants observed by Wang and the group [74]. Volatile constituents of *G. lemaniformis* also possesses antioxidant activities since *in vitro* biochemical assays such as hydroxyl radical, DPPH and superoxide radical scavenging assays performed by Yuan et al. [75] demonstrated this evidence. *G. lemaniformis* extract derived antioxidant activities through SOD, CAT and POD mediated was also reported by many other groups [76].

Antimicrobial properties: Due to uncontrolled use of antibiotics in fisheries and aquaculture sectors that causes pathogen resistance to fish diseases, scientists are looking for alternative natural and non-hazardous origin of antibiotic mimics and marine origin is the vast sector. Many reports suggest that seaweed extracts demonstrated strong antimicrobial activities [77,78]. Bioactive compounds from seaweed extracts, such as steroids, alkaloids, polysaccharides, and fatty acids, not only inhibited pathogens, but also improved the immunity of animals as well as improved disease resistance [79]. Based on previous findings, bioactive compounds from seaweeds increased THC, proPO, SOD, and lysozyme activities and other immunological parameters of shrimps, thereby improving disease resistance [80,81]. *G. lemaniformis* extract showed highly potent antimicrobial activity against S. aureus, and *V. alginolyticus* in a disc diffusion method, and also enhanced the immune response and reduced the mortality rate of WSSV-infected crabs [82]. Another report showed antibacterial activity of the hot water extract of *G. lemaneiformis* against *S. aureus* and *E. coli* [78]. Invertebrates such as crabs lack immunoglobulin in body fluids, and mainly rely on non-specific innate immune mechanisms to resist pathogenic invasions. *G. lemaneiformis* enhanced the innate immunity of healthy crabs [82].

Antitumor or anticancer properties: Many of the bioactive compounds like polysaccharides found in marine algae have been extensively studied as anticancer agents. Algal polysaccharides have great pharmacological importance. Due to the structure and activities of some active polysaccharides in *G. lemaniformis*, they are considered as active antitumor agents. Polysaccharides having such structural similarities found in G. lemaniformis are 3,6-anhydro-L-galactose, D-galactose and some acidic polysaccharides with a linear structure of repeated disaccharide agarobiose units [83-86]. *G. lemaniformis* had potential antitumor activity and in a study, it was observed that *G. lemaniformis* extract showed strong antitumor activity through apoptosis-related Fas/FasL signaling pathway in the gastric cancer cell line MKN28, human lung cancer cell line A549 and mouse melanoma cell line B16. *G. lemaniformis* dramatically inhibited the cancer cell growth of these human and mouse cancer cells [87]. This same group in a previous study using transcriptomic data analysis using the *G. lemaniformis* extracts in three human cancer cell line showed that 758 genes expression related to apoptosis are regulated by this species [88]. In another study, it was reported that *G. lemaniformis* aqueous extracts-based antioxidant and antitumor activities were diminished or decreased by the exposure of TiO_2 nanoparticles in HepG2 cells. In that study, 3.3-fold increased malondialdehyde level and decreased level of superoxide dismutase and catalase activity were observed upon exposure to TiO_2 nanoparticle, which leads that TiO_2 nanoparticle has cancer cell proliferation effect and suppressing effect on *G. lemaniformis* extract mediated antitumor effect [89].

Polysaccharides from *G. lemaniformis* showed enhanced anticancer efficacies when it was applied to Glioblastoma multiforme (GBM), the most lethal primary brain tumor in human, along with nanoparticles. The poor permeability of glioma parenchyma is the burning issue for antiglioblastoma drug delivery and selenium nanoparticle (SeNPs) is the best choice to treat such type of fatal disease. Unmodified SeNPs drug delivery efficacy is very poor, but a satisfactory level of drug delivery was recorded from a modified SeNPs with *G. lemaniformis* derived polysaccharide (GLP-SeNPs). GLP-SeNPs reveals higher cellular uptake of this drug rather than the SeNPs alone in U87 cells [90]. As GLP has strong apoptotic ac-

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tivity by upregulating apoptosis pathway (activated p53, MAPKs, and AKT pathway), so that GLP-SeNPs drug delivery system's additive efficacy is governed by GLP. Besides, dietary fibers from *G. lemaniformis* also possesses antitumor activity [91]. Proteomic and bioinformatic analysis also reveals antitumor activity of *G. lemaniformis* species. Study showed that phycoerythrinm, an active bioactive compound isolated from *G. lemaniformis* exerts significant antitumor activity in human SW480 cell. With IC50 value of 48.2 ug/ml, phycoerythrin induced apoptosis and cell cycle arrest in SW480 cells with decreased level of GRP78, NPM1, MTHSP75, Ezrin, Annexin A2 and increased level of HSP60, which demonstrates that *G. lemaniformis* derived phycoerythrin has antitumor activity [92]. Beside these, bunch of reports suggests the anticancer role of *G. lemaniformis* seaweed [93-95].

Antidiabetic activity: Polysaccharides from *Gracilaria lemaneiformis* can improve both specific and nonspecific cellular immune responses through immune-regulatory pathway [93-95]. *G. lemaniformis* extract also possesses antidiabetic properties. In alloxan-induced diabetic model mice, it was reported that 21 days consecutive administration of the seaweed extract significantly lowers the blood glucose level by inhibiting alfa-glucosidase activity. It also provided pancreatic and renal cell repair or protection from alloxan-induced damage [96]. Another report also suggested that *G. lemaniformis* exerted its antidiabetic effect in streptozotocin-induced model mice over metformin treatment [97].

Unsaturated fatty acid source: Unsaturated fatty acids are beneficial to health because they can improve blood cholesterol levels, stabilize heart rhythms, ease inflammation and so on. Studies suggested that *G. lemaniformis* contains substantial amount of unsaturated fatty acids. Both saturated and unsaturated fatty acids are observed in this species. Among the saturated fatty acids, the predominant one was palmitic acid (16:0) and as a predominant unsaturated fatty acid oleic acid (18:1) was reported [98].

Conclusion and future prospect

Since it has been discussed earlier that due to the population burden, the increased population demands more food, nutrition, medicine, and other health related support, so that, to compensate these demands, scientists are looking for alternative resources rather terrestrial areas in the world [99]. Ocean would be the alternative vital resource due to around 71% coverage of the earth. At present most of the cancer treatment drugs are originated from the marine sources and in many cases, seaweeds satisfy this demand. As *G. lemaniformis* also has food values with rich contents of proteins, fibers, minerals, polysaccharides, and vitamins, along with its pharmacological values, so that in near future this species would be helpful for the development of new therapeutic drugs.

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