



SEA GRASSES - NOVEL MARINE NUTRACEUTICALS

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ABSTRACT

Marine plants are known to produce a large number of structurally diverse secondary metabolites. They are found to be economically important in the field of food additives, nutraceutical and drugs. Seagrasses are a paraphyletic group of marine hydrophilus angiosperms, which evolved three to four times from land plants back to the sea. It lives in an estuarine or in the marine environment, and nowhere else. Seagrasses contain several compounds in their secondary metabolism in which they differ from terrestrial plants. They produce novel chemicals to withstand extreme variations in pressure, salinity, temperature, and so forth, prevailing in their environment, and the chemicals produced are unique in diversity, structural, and functional. The phytochemical present in seagrasses exhibit antibacterial, antioxidant, antitumor activity. New inclination in the field of drug discovery from natural sources highlights on the investigation of the marine ecosystem to survey numerous complex and novel chemical entities. These entities are the source of new lead form treatment of many diseases such as cancer, AIDS, inflammatory condition, arthritis, malaria and large variety of viral, bacterial, fungal diseases. Several species of sea grasses are used as human food or as raw material for the production of compounds of nutritional interest. Compared to algae, sea grasses remain less exploited despite the fact that they offer tremendous opportunities to find new commercially valuable phytochemicals. Therefore, their metabolite contents constitute another treasure of the ocean which is hidden. This paper gives an overall view on their nutraceutical activity and their potential as anticancer agent so that the hidden facts will be explored to provide a new cheap source for therapeutically and nutraceutical application. Thus, it is evident from various literature that sea grasses could be used as a potential source for natural health product.

KEYWORDS: Phytocompounds, Anti-fouling, Antioxidant, Halophytes, Zostrine, Lignins.



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INTRODUCTION

Sea grasses are flowering plants that grows in a marine fully saline environment forms dense underwater meadows. They were found in shallow salty and brackish water in many parts of the world from the tropics to the Arctic Circle. Sea grasses evolved 100 million year ago and today there are approximately 72 different species that belongs to four major groups. Among these species thirteen are found in the Tamil Nadu state, including: *Enhalus acoroides*, *Halophila beccarii*, *Halophila decipiens*, *Halophila stipulacea*, *Halophila ovalis*, *Halophila ovalis* ssp. *ramamurthiana*, *Halophila ovata* Gaud., *Thalassia hemprichii*, *Cymodocea rotundata*, *Cymodocea serrulata*, *Halodule pinifolia*, *Halodule uninervis*, and *Syringodium isoetifolium*.¹ Several species of this group produce antimicrobial compounds that may act to reduce or control microbial growth. They have long been recognized as producer of biologically active substances and it has been suggested that sea grasses produce secondary metabolites that have a defensive role against marine pathogens. In traditional medicine, they have been used for a variety of remedial purposes, e.g. for the treatment of fever and skin diseases, muscle pains, wounds and stomach problems, tranquillizer for babies, and also as remedy against stings of different kinds of rays etc. This document tells about their unique phytochemicals present in them and about their nutraceutical activity that has been explored.

POLYPHENOLS

Polyphenols are especially important antioxidants, with high redox potentials, which allows them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. Sea grasses contain high amount of polyphenols such as phenolic acids, flavonoids, anthocyanidins, lignin, tannins, catechin, epicatechin, epigallocatechin, and gallic acid.² These polyphenolic compounds have shown many health-benefiting bioactivities, such as antioxidant, anticancer, antiviral, anti-inflammatory, and an ability to inhibit human platelet aggregation. Some studies have shown a positive correlation between the increased dietary intake of natural antioxidants and the reduced coronary heart disease, cancer mortality, as well as longer life expectancy.³ Moreover, they are natural metal chelators with high antioxidant activity that may be successfully used to prevent a variety of toxic metal ion-induced

organ dysfunctions. Earlier reports suggest that polyphenols may regenerate α -tocopherol through reduction of the α -tocopheryl radical. A close association between anticarcinogenic activity and antioxidant activity has been reported in a chemically induced mouse carcinoma system with low-molecular weight polyphenols.³ Phenolic compounds were isolated from *Zostera marina*, a species of sea grass commonly called as eelgrass, and suggested to have an important role in inhibition of microbial growth, amphipod grazing and in the resistance to the so-called waste-disease.⁴ The fact that these crude methanolic extracts of *Zostera marina* have been found to inhibit the attachment of marine bacteria, diatoms, barnacles, and polychaetes on artificial surfaces suggests an antifouling potential of *Zostera marina* phenolic compounds. The p-(sulfoxy) cinnamic acid (zosteric acid) was isolated for the first time as a natural product from the seagrass *Zostera marina* and was found to prevent attachment of marine bacteria and barnacles to artificial surfaces at nontoxic concentrations. At least 23 phenolic compounds were identified in the sea grass *Posidonia oceanica*.⁵ Gallic acid is synthesized and stored in more than 50% of all seagrass species. Chicoric acid and caftaric acid were identified in detrital and living leaves of the tropical seagrass *Syringodium filiforme* making this abundant renewable raw material of interest for pharmaceutical purposes and food industries. Phytochemical investigations revealed the presence of unidentified sulfated phenolic compounds from nine different species of *Halophila*, unidentified sulfated and non-sulfated flavones from the *Halophila ovalis*/*Halophila minor* complex, flavones and flavone glycosides from *Halophila johnsonii*.⁶ as well as malonylated flavonoid derivatives in the seagrass *Halophila stipulacea*.⁷ Production of phenolic compounds depends on the environmental conditions. For example, tannin production can be wound-induced in *Thalassia testudinum* under simulated grazing conditions, and the content of phenolic compounds in shoots of *Zostera marina* varied seasonally. The concentration of phenolic compounds was measured in the seagrass *Posidonia oceanica* when interacting with two Bryopsidophyceae, *Caulerpa taxifolia*, and *Caulerpa racemosa*.¹² Several phenolic compounds were identified in *Posidonia oceanica*, with a predominance of caffeic acid in the adult and intermediate leaves.⁸ On examining phytochemicals of *Halophila ovalis*, *Syringodium isoetifolium* and *Halodule pinifolia* show the presence of phenolic compound which exhibits antioxidant property.

HALOPHILA OVALIS



SYRINGODIUM ISOETIFOLIUM



HALODULE PINIFOLIA

Figure 1
Digital images of *Halophila ovalis*, *Syringodium isoetifolium* and *Halodule pinifolia*

Research on sea grasses proved the presence of lignin in several tissue types and several isoblastic cells. Species variation of the lignin content is dependent on the morphotype and life style of the seagrass. *Posidonia oceanica* contains more lignin than *Zostera marina*, presence of lignin in sea grasses shows their defense mechanism against microbial attack. The importance of lignin in making seagrass below-ground organs particularly decay-resistant still needs to be adequately addressed as well as their role as carbon sink. In comparison to lower plants species the seagrass

FLAVONOIDS

The presence of sulfated flavones has been reported in *Halophila*, *Thalassia*, and *Zostera* species, but they were not recorded in *Syringodium* spp. or *Posidonia oceanica*. Flavonoid sulfates were also detected in *Halophila ovalis* and *Thalassia testudinum*.⁹ The four flavones luteolin, apigenin, luteolin-3-glucoronide, and luteolin-4-O-glucoronide, with anti-microbial potential

have been identified from the ethanol extract of air-dried *Enhalus acoroides* as shown in figure 2 from South China Sea reported flavonoid glycosides and acyl derivatives, which yield after hydrolysis the respective flavonoid aglycones in *Posidonia oceanica* leaves. Later, they have found dramatic losses of flavonoids when analyzing freeze-dried and chilled leaves as opposite to fresh and oven-dried leaves of *Posidonia oceanica*.¹⁰ Bitam et al isolated and identified malonylated flavone glycoside derivatives from *Halophilastipulacea* using HPLC and NMR.¹¹ Heglmeier and Zidorn compiled and appraised the data of secondary metabolites of *Posidonia oceanica* and they summarized 51 natural products including phenols, phenylmethane, phenylethane, phenylpropane derivatives and their esters, chalcones, and flavonoids.¹² Significantly higher flavonoid amounts were observed in the leaves of intertidal and subtidal *Halophilajohnsonii* when compared to the leaves of intertidal *Halophiladecipiens*. These functional derivatives of flavonoids are considered to strive against the marine microorganisms exhibiting chemical defense.

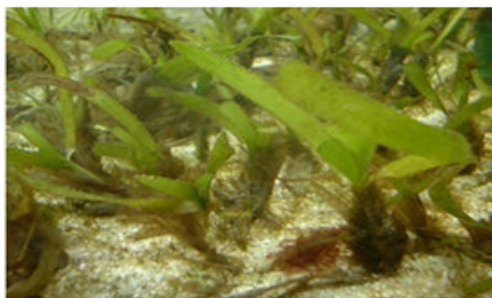


Figure 2
ENHALUS ACORIDES

TERPENOIDS

Molecules found in terrestrial plants such as caffeic acid, inositol, sucrose, monoglucoside of quercetin, monoglucoside of isoramnetin, cichoric acid, as well as polyamines like putrescine, spermidine, and spermine have been reported as constituents of *Cymodocea nodosa*. Furthermore, 24a-ethyl sterols and 24a-methyl sterols along with their 24b-epimers, cymodiene and cymodienol, the first diarylheptanoids isolated from marine organisms, comprise the total number of

metabolites isolated from *Cymodocea nodosa* so far. Recently, new terpenoid compounds from the structural class of diarylheptanoids, a new meroterpenoid, and the first briarane diterpene isolated from seagrass, and only the second analog of this class with a tricyclic skeleton. Furthermore, this metabolite is the first brominated briarane diterpene.¹³ All newly detected compounds were assayed for their antibacterial activity against multidrug resistant (MDR) and methicillin-resistant bacterial strains. The activity reached from weak to strong and therefore opens the field for the formulation of new antibiotics which are urgently needed due to rise

of multidrug resistant strains, especially in hospitals.¹³ Probably a large number of compounds with potent antibiotic activity can be extracted from sea grasses. The chemical and biological investigations of chloroform extract from *Posidonia oceanica*, a marine phanerogam as shown in figure 3 has led to the isolation and identification

of a novel methylated sesquiterpene: (E)-3, 7, 12-trimethyltridec-2-en-1-ol named posidozinol along with sitosterol and four known fatty acids: palmitic, palmitoleic, oleic and linoleic acids.¹⁴ Terpenoids isolated from *Posidonia oceanica* demonstrated good antifungal activity.



Figure 3
POSIDONIA OCEANICA

POLYSACCHARIDES

Glucose, galactose, and mannose were the dominant sugars (>10 g kg⁻¹ of dry matter) in the soluble fraction of nonstarch polysaccharides in all seagrass forms. The content of the insoluble constituents of the nonstarch polysaccharides was significantly higher than soluble nonstarch polysaccharide constituents ($P < 0.01$). Data showed that the major constituents of the *Posidonia* cell wall are cellulose and lignin (190-209 and 145-154 g kg⁻¹, respectively).¹⁵ Carbohydrates of sea grasses evolved differently from terrestrial plant, leading to a broad range of different inositols in addition to the ubiquitous sucrose, glucose, and fructose. Of the nine possible inositols, five are known to occur in plants; myo-, l-chiro-, muco- and O-methyl-mucoinositol occur in seagrasses. Leaves and rhizomes of some seagrasses, particularly the *Zosteraceae*, contain relatively large amounts of myo- inositol, up to a maximum of 2.2% dry weight in *Zostera noltii* rhizomes. In most plants the cyclization enzyme is conservative and yields only myo-inositol, which then acts as the sole precursor for many other inositols. Drew suggested that, since the configuration of the glucose molecule would permit the direct formation during cyclization of all the inositols found in seagrasses, they may be inevitable byproducts of another, less specific, glucose cyclization enzyme.¹⁶

SULPHATED POLYSACCHARIDES

Seagrass is subject to extremely salty conditions, and it's had to adapt to osmotic stress. Unlike typical plant cell walls, seagrass has engineered its cell wall matrix to retain water in the cell wall, even during low tide. This involves depositing sulfated polysaccharides and low methylated pectins in the cell wall matrix, but until its genome was sequenced no-one knew exactly how. It turns out that seagrass has rearranged its metabolic pathways.¹⁷ Sulphated polysaccharides (SP) of seagrass species are composed of galactose units. Seagrass species contain various amounts of this compound depending on the parts of the plant and on the salinity of their environment (*Halodule wrightii* 8.5 g SP and *Halophiladecipiens* 7.7 g SP per mg dry weight), comparably high as some mangrove species, whereas in terrestrial crop plants the values are below 0.001 g SP per mg dry weight.¹⁷ The sulphated polysaccharides synthesized from *Ruppia maritima* as shown in figure 4 was purified and its structure was suggested. It is a sulfated homogalactan composed of a regular repeating unit: [3-β-D-Gal-2(OSO₃)-1→4-α-D-Gal-1→4-α-D-Gal-1→3-β-D-Gal-4(OSO₃)-1→]. This SP was found in the plant cell walls, mostly in rhizomes and roots. Since salt intolerant plants do not contain SP, it has been suggested that there is a possible correlation between the presence of these compounds and seagrass salt tolerance.¹⁸



Figure 4
RUPPIA MARITIMA

Zosterin, an apiose-rich pectic polysaccharide, was extracted and purified from the sea grass *Zostera marina* as shown in figure 5. Structural studies conducted by gas chromatography and NMR spectroscopy on a purified zosterin fraction (AGU) revealed a typical apiogalacturonan structure comprising an α -1,4-d- galactopyranosyluronan backbone substituted by 1,2- linked apiofuranose oligosaccharides and single apiose residues. The average molecular mass of AGU was estimated to be about 4100 Da with a low polydispersity. AGU inhibited proliferation of A431 human epidermoid carcinoma cells with an approximate

IC(50) value of 3 $\mu\text{g/mL}$ (0.7 μM). In addition, AGU inhibited A431 cell migration and invasion. Preliminary experiments showed that inhibition of metalloproteases expression could play a role in these anti-migration and anti-invasive properties. Auto hydrolysis of AGU, which eliminated apiose and oligo-apiose substituents, led to a virtual disappearance of cytotoxic properties, thus suggesting a direct structure-function relationship with the apiose-rich hairy region of AGU. From these details we come to know that sulphated polysaccharides is unique to each sea grasses.¹⁹



Figure 5
ZOSTERA MARINA

DIMETHYLSULFONIOPROPIONATE (DMSP)

Seagrasses produce dimethylsulfoniopropionate as a osmoprotectant, much of which is degraded by marine bacteria as dimethyl sulphide, ensures an important role for both compounds in the global sulfur cycle. In seagrasses different DMSP concentrations have been found: *Halodule wrightii* 3.3 mol g^{-1} fresh weight, *Syringodium filiforme* 0.10 mol g^{-1} fresh weight, *Thalassia testudinum* in epiphytized and nonepiphytized leaves between 0.18 and 4.0 mol g^{-1} fresh weight, and very low amounts in the rhizome.²⁰ These results indicate that the degree of epiphytization plays a major role in the contribution of seagrasses to the total DMSP production. The regulation of the biosynthetic pathway of DMSP in seagrasses needs to be elucidated to clarify the overall contribution by seagrasses.

PROTEINS AND PEPTIDES

Seagrass has a unique nature to absorb heavy metals from their surroundings and to accumulate them in different tissues in different extent. This absorption and accumulation is mediated by proteins and peptides. PCs and metallothioneins (MTs) are Cys-rich metal chelators that represent the two principle groups of metal-binding molecules found across most taxonomic groups.²¹ PCs, glutathione-derived metal binding peptides, usually with the structure of -Gly (-11) are enzymatically synthesized peptides known to be involved in heavy metal detoxification, mainly Cd and As, which has been demonstrated in plants, algae, and some yeast species grown at high heavy metal concentrations.²² So far, neither the exact composition of PCs in sea grasses nor

the biosynthetic enzyme, PC synthase, have been analyzed in seagrasses. MTs are a group of proteins with low molecular mass and high cysteine content that bind heavy metals and are thought to play a role in their metabolism and detoxification. The criteria that define a protein or peptide as an MT are (i) low molecular weight (<10 kDa), (ii) high metal and sulfur content (>10%), (iii) spectroscopic features typical of M-S bonds, and (iv) absence or scarcity of aromatic amino acids. However, when all criteria are not fulfilled, the proteins are often called as MT-like proteins. There are only a few articles reporting on the metal-binding mechanisms of sea grasses.²² Three genomic sequences putatively encoding MTs were isolated from *Posidonia oceanica*, namely, Pomt2a, Pomt2b and Pomt2c that showed high similarities to putative plants MTs. Pomt2a and Pomt2b contain a CXXC motif classifying them as type II plant MTs, the remaining Pomt2c is considered to be a pseudogene. Moreover, authors indicated that there were at least five MT genes present in *Posidonia oceanica* genome based on Southern blot hybridizations. Results of Giordani et al showed that based on Northern blot hybridization MT transcript accumulation was increased by Cu and Cd exposure, whereas no apparent effect was observed after Hg treatment. Higher Cu^{2+} concentration (10 mol) treatment showed higher MT transcript accumulation than low Cu^{2+} concentration (1 mol).²³ Based on these results Cozza et al continued to carry out the studies in more detail. Nine MT-like sequences from Cu or Cd treated *Posidonia oceanica* were isolated by RT-PCR. One sequence is similar to Pomt2b. Phylogenetic analysis of MT-like protein deduced from isolated MT-encoding genes from *Posidonia oceanica* showed two subgroups. To better understand the functional role of the two MT subgroups one gene representative for each group was used for in situ hybridization to discover

spatial expression of the plant. Interestingly, the members of these two MT subgroups showed differences in their histological expression, with Pomt2b associated with the proliferative tissues whereas Pomt2f was associated with the lignified or suberized cell wall.²⁴

CONCLUSION

Phenolic compounds like chicoric acid from *syringodium filiforme*, gallic acid from 50% of all sea grasses, and zosterin from *zostera asiatica* which is already in application as anti-tumour drug provides evidence for the usage of seagrass as a nutraceutical. Free L-chiro-inositol was isolated from aqueous extracts of dried detrital *Syringodium filiforme* leaves. The high concentrations found (2.3–2.5% dry weight) offer promise for the exploitation of *Syringodium flotsam* as a new cheap source for nutraceutical or therapeutic

applications, considering the demonstrated hypoglycaemic action of L-chiro-inositol. Unique nature of seagrass such as heavy metal chelating peptides and proteins and DMSC establish its nutraceutical application. One of the rare real applications seems to be zosterin, a bioactive pectin from *Zostera asiatica*, which decreases toxicity of antitumor drugs and purges heavy metals from human organisms.²⁵ These properties led to a patented and marketed drug and food in Russia (Patents RU2128918, RU2129388C1, RU2132696C1, and RU2242217). Owing to a diverse chemical ecology, the marine organisms have a great promise for providing potent, cheaper, and safer anticancer drugs, which deserve an extensive investigation.

CONFLICT OF INTEREST

Conflict of interest declared none.

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