

PHYTOCHEMISTRY AND ETHNOPHARMACOLOGY OF THE SEAGRASS CYMODOCEACEAE FAMILY – A REVIEW

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ABSTRACT

Cymodoceaceae is a family of flowering plants, sometimes known as the “manatee-grass family,” the family Cymodoceaceae includes only marine species. The angiosperm phylogeny II system, of 2003 (unchanged from the APG system, of 1998), does recognize Cymodoceaceae and places it in the order Alismatales, in the clade monocots. They are marine hydrophytes that grow and complete their life cycle in a submerged condition, in a saline environment. Like terrestrial plant they obtain their energy from light through photosynthesis thus, they grow only in clear and shallow water, and at the suitable condition, they form beds or meadows. The family includes five genera, totalling 16 species of marine plants occurring in tropical seas and oceans (so-called seagrasses). Cymodoceaceae consist of five genera such as *Amphibolis*, *Cymodocea*, *Halodule*, *Syringodium*, and *Thalassodendron*. In this genera *Cymodocea rotundata* Ehrenb. and Hempr. Ex Asch. *Cymodocea serrulata*, (R.Br.) Asch. and Magnus, *Halodule pinifolia* (Miki) Hartog, *Halodule uninervis* (Forssk.) Asch and *Syringodium isoetifolium* (Asch.) are the species mostly adopted in Indian coastal region. These seagrass species have unique nature and wide application to the environment including human being. In this article botanical aspects, phytochemistry and ethnopharmacology of these five seagrass species belong to Cymodoceaceae family will be discussed.

Keywords: Cymodoceaceae, Seagrass, *Cymodocea*, Ethnopharmacology, Phytochemistry, Hydrophytes, *Syringodium isoetifolium*, *Halodule*.

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INTRODUCTION

The more complex ecosystem in our earth is a marine ecosystem, which is vast and yet to be explored. In marine environment, seagrass is a primary producer where fishes and most of the marine organism dwells and feeds on the seagrass beds. Thus, they form the bottom line of the food chain. Seagrasses are flowering plants belongs to order Alismatales that complete their entire life cycle under sea water in a saline environment this unique nature such as withstanding high salinity and high water pressure makes them to produce phytochemicals that have medicinal value. Seagrasses are divided into four families such as Posidoniaceae, Zosteraceae, Hydrocharitaceae, and Cymodoceaceae under order Alismatales and class of monocotyledons. There are 12 genera with some 60 species known. Among these family Cymodoceaceae purely belongs to the marine organism and they are highly diverse consist of five genera such as *Amphibolis*, *Cymodocea*, *Halodule*, *Syringodium*, and *Thalassodendron* [1]. In *Cymodoceaceae*, *Cymodocea rotundata* Ehrenb. and Hempr. Ex Asch., *Cymodocea serrulata*, (R.Br.) Asch. and Magnus, *Halodule pinifolia* (Miki) Hartog, *Halodule uninervis* (Forssk.) and *Syringodium isoetifolium* (Asch.) are the species mostly adopted in Indian coastal region [2]. They are widespread in a place where it is growing and can be easily recovered from cyclone disturbance. This seagrass has been used food and medicine by the ancestor of the coastal indigenous society. The root of *Cymodocea* is eaten as food commonly known as sea sugarcane. Some of *Cymodocea* species are used against malaria, cough and also used as tranquilizers for babies [3]. This document revises the botanical aspects, phytochemistry, and ethnopharmacology of seagrass family Cymodoceaceae that are adopted in Indian coastal region.

BOTANICAL ASPECTS

Seagrass is highly diverse species though they are similar to each other there are botanical aspects that can differentiate them.

Habitat

Since seagrass gets energy through photosynthesis, they grow in clear, shallow water. At suitable environment, they grow rapidly and spread easily forming sea meadows or sea beds where the ecosystem is created.

Seagrasses are not grass like terrestrial *Poaceae*, they were called so because of their ribbon-like grassy leaves. They are flowering plants with roots, stem, leaves, flower, and seeds. There is even a type of seagrass that does not look like a grass. Seagrasses are more closely related to terrestrial lilies and ginger than to true grass. They grow, produce flowers and seeds. Their seeds spread through water and start germination producing new plant and meadows. Fig. 1 shows the seeds produced by seagrass; they will disperse in water and spread into a new plant. Seagrasses require inorganic carbon for growth, nutrients were absorbed by the roots and uptake by leaves surface by two pathways which are species specific.

S. isoetifolium and *C. rotundata* use bicarbonate (HCO_3^-) as an inorganic carbon whereas *Halodule*, *C. serrulata* use an enzyme to convert carbon dioxide into the available inorganic carbon source [4].

Taxonomy

Kingdom: Plantae - plants
 Subkingdom: Tracheobionta - vascular plants
 Super division: Spermatophyta - seed plants
 Division: Magnoliophyta - flowering plants
 Class: Liliopsida
 Subclass: Alismatidae
 Order: Najadales
 Family: Cymodoceaceae.

Morphology

Seagrass like terrestrial plant their morphology divides into veins, stems, roots, and reproductive parts. Fig. 2 shows the morphological variation among its taxonomy.

Leaf

Leaves of seagrass vary from tiny fingernail size too long as 7-meter size. Seagrass *Halophila* has oval or paddle shape or fern shape leaves, *Cymodocea* and *Halodule* have ribbon shape leaves, *Syringodium* has long spaghetti like leaves. Their leaves lack stomata instead they consist of thin cuticle to exchange gas and nutrients. Each seagrass differs in their leaf morphology is shown in Fig. 3.



Fig. 1: The seeds of seagrass

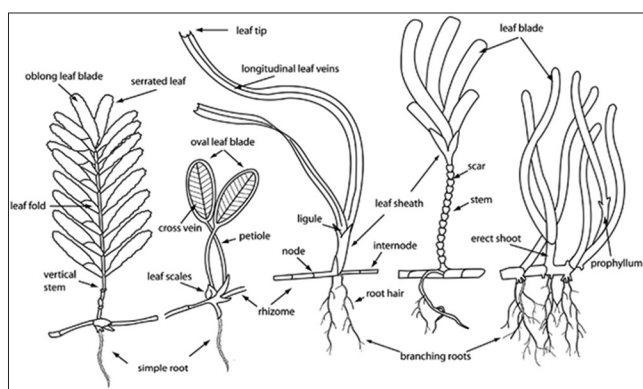


Fig. 2: The morphological features used to distinguish main seagrass taxonomic groups

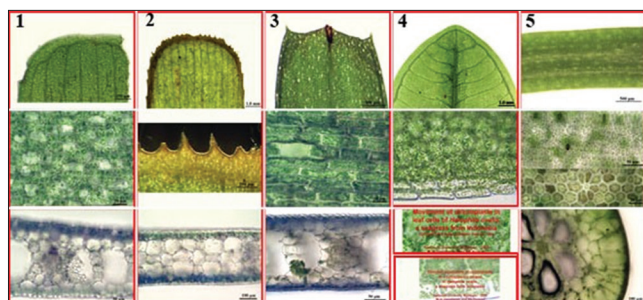


Fig. 3: The morphology of leaves (1) *Cymodocea rodunta*, (2) *Cymodocea serrulata*, (3) *Halodule universi*, (4) *Halophilus ovalis*, (5) *Syringodium isoetifolium*

Rhizome

They consist of roots that were buried in the sediment on the sea floor, such a way it can anchor the plant against the forces of wave action and tidal currents. This root-like structure called rhizome absorbs nutrients from the sea. They may be simple or branching formed in segments with leaves or vertical stems. They consist of nodes and internodes where the leaves and stems will be formed.

Reproductive organs

Seagrasses are hydrophilic in reproduction. They produce flowers which undergo pollination and reproduce when they are submerged and complete their entire life cycle under water. Their pollen is elongated into filamentous shapes that facilitate them to transport within water.

Adaptation

The survival of seagrass depends on light, nutrients, carbon dioxide and substrate for anchoring, tolerable salinity, temperature and pH of their environment. Alteration in these parameters will directly affect the

population of seagrass. Adaptation of seagrass to marine environment subject them to major constraints on their morphology and structure thus there is variation among species of seagrass such as *Cymodocea* prefers deeper sediments while others can tolerate a broad range of sediments depths. *H. uninervis* are better suited to mobile sediments since they are colonizing seagrass. The highest concentration of seagrass species occurs in an Indo-west Pacific region which might be suitable adaptation region for it. Table 1 lists the morphology of Cymodoceaceae species that were grows in Indian coastal region.

PHYTOCHEMISTRY

To withstand extreme variations in pressure, salinity, temperature these seagrass produce unique metabolites that differ from terrestrial plant. Their biochemical adaptation evolved in such a way to protect themselves from the competitive environment which is responsible for their molecular thermal-adaptations. Lipids and fatty acid composition of seagrass might be responsible for their thermal stability [5]. Chlorophyll content of seagrass is an interesting factor since they vary according to the season. It has been reported that during monsoon season total chlorophyll and chlorophyll a and b contents are lower and during summer season they are higher. This variation is due to the light stress they were undergoing because of the water turbidity. Chlorophyll content also varies among species thus their leaf morphology also differs according to it. *H. uninervis*, *H. pinifolia*, and *S. isoetifolium* have lower chlorophyll content grows in deeper water area whereas *Halophila ovalis* has higher chlorophyll content with broader leaf. Like leaf morphology, rhizome morphology also varies among species this is due to the carbohydrate content present in it. Seagrasses are rich in carbohydrates there is higher carbohydrate content in root than the leaf. Soluble sucrose and other soluble carbohydrate such as glucose and fructose are in higher content [6]. Table 2 lists the photochemical present in five species of Cymodoceaceae those are predominant in Indian coastal area. Calorific value of selective seagrasses was determined and compared to vegetables by Pradheeba *et al.*, according to them, *C. rotundata* was comparable to that of potato and sweet potato.

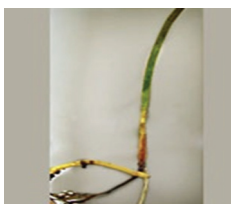
Polyphenols

Since seagrass has to undergo various stress, they were rich sources of phenolic acids, sulfated phenolic acids, flavones, condensed tannins, flavonoids and also lignins [12]. The phenolic compounds play an important role in the protection of seagrasses against competitors, predators, and pathogens. Production of phenolic compounds depends on the environmental conditions. For example, tannin production can be wound-induced in *Thalassia testudinum* under simulated grazing conditions [13]. Soluble phenolic acids shown to be abundant in a variety of seagrasses and their extracts are rich in soluble phenols which have been found to inhibit the growth of bacteria, fungi, and algae. *Halodule* Spp., *Cymodocea* Spp., *S. isoetifolium* consist of a phenolic acid such as p-coumaric acid, caffeic acid, ferulic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, gentisic acid, and Gallic acid [14]. At most 50% of all seagrass species produce and store Gallic acid [15]. A Gallic acid derivative (octyl gallate) shows a strong antimalarial activity by inhibiting the receptor of malarial dihydrofolate reductase [16]. Seagrasses such as *C. serrulata*, *H. pinifolia*, and *H. uninervis* were rich in flavonoids. These flavonoids contain chemical structure that has an ability to scavenge free radicals thus they can act against to various diseases that were caused by oxidative stress [17]. According to the epidemiology study intake of flavonoids reduces the mortality rate of coronary heart disease and heart attack. The 50-800 mg of flavonoids is a significant total daily intake as they took part in the antioxidant defense system [18]. The presence of sulfated flavones is a unique nature of seagrass however they were not present in *Syringodium* sp. but each of the six genera of *Cymodoceoideae* and *Posidoniodeae* had two or three sulfated phenolic acids [19].

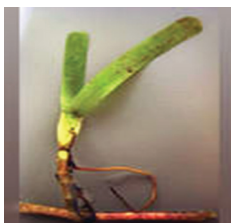
Sulfated polysaccharides

Polysaccharides composition of seagrass varies from the terrestrial plant may be due to osmoregulation. In addition to existing sucrose,

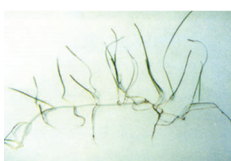
Table 1: Morphology of Cymodoceaceae seen in Indian coastal region

C. rotundata

Flat, strap-like leaves 2-4 mm wide
Rounded, smooth leaf tip
Smooth rhizome
Scars from well-developed leaf sheaths form a continuous ring around the stem
Found on shallow reef flats

C. serrulata

Linear strap-like leaves, 5-9 mm wide
Serrated leaf tip
Leaf sheath is broadly triangular with a narrow base
Leaf scars do not form a continuous ring around the stem
Found on shallow subtidal reef flats and sand banks

H. pinifolia

Fine, delicate leaves up to 20 cm long
1 central vein
Black central vein splits into two at the rounded leaf tip
Usually pale rhizome, with clean black leaf scars
Found on intertidal sand banks

H. uninervis

Usually larger than *H. pinifolia*
Trident leaf tip
1 central longitudinal vein
Rhizome usually pale ivory, with clean black leaf scars
Dugong preferred food
Found on shallow/intertidal sand or mud banks

S. isoetifolium

Cylindrical in cross section (spaghetti like)
Leaf tip tapers to a point
Leaves 7-30 cm long
Found on shallow subtidal reef flats and sand banks

C. rotundata: *Cymodocea rotundata*, *C. serrulata*: *Cymodocea serrulata*, *S. isoetifolium*: *Syringodium isoetifolium*, *H. pinifolia*: *Halodule pinifolia*, *H. uninervis*: *Halodule uninervis*

glucose, fructose they consist of a broad range of inositols. Among nine inositols that occur naturally five inositols such as Myo-, chiro-, muco-, o-methyl-muco-inositol were found in seagrass. In Cymodoceaceae all genera except *Halodule* contains these five inositols with l-chiro-inositol predominantly at most 6.8% dry weight [20]. Muco-inositol was less widely distributed. The presences of these sulfated polysaccharides facilitate seagrass to transport ion at high salt concentrations. These sulfated polysaccharides are galactose in seagrass and they were four sulfation.

Metal chelating

The unique nature of Cymodoceaceae is absorbing heavy metals from sea water. They taken up the metals and accumulated it in their different tissues in different concentration. Thus they act as a bio-indicator for heavy metals to differentiate contaminated areas from non-contaminated areas of the sea. The study was done by Thangaradjou *et al.* shows that the concentration of the four toxic heavy metals (Cd, Pb, Zn, and Cu) was found higher in all the seagrasses when compared with the background values of seagrasses from Flores Sea, Indonesia [21]. The contamination factor of these four heavy metals ranged as Cd (1.97-12.5), Cu (0.73-4.4), Pb (2.3-8.89),

and Zn (1.27-2.787). In general, they also noticed that small leaved seagrass such as *H. uninervis* and *H. pinifolia* can be efficiently absorb metals than other seagrass. It also suggested that the phytochelatin and metallothioneins are Cys-rich metal chelators that represent the two principal groups of metal binding molecules found across most taxonomic groups [22]. However, there is no evidence of these metal chelating peptides or proteins were present in the extract of seagrass of Cymodoceaceae. Further researches were needed to study metal chelating activity of these seagrasses.

ETHNOPHARMACOLOGY

Information on traditional use of plants, especially on its pharmacological effects is called as ethnopharmacology. Ethnopharmacology of plant is very important for the discovery of novel drug from natural products [23]. In traditional medicine, seagrass has been used for a variety of remedial purposes, e.g., for the treatment of fever and skin diseases, muscle pains, wounds and stomach problems, tranquilizer for babies, and also as remedy against stings of different kinds of rays etc., Table 3 shows the ethnopharmacology use of five species of Cymodoceaceae that are abundant in Indian coastal region.

RECENT APPROACHES

On the basis of their traditional uses, for the past decades, there has been attention toward marine angiosperm on discovering their potential phytochemicals and their secret behind adaptation. Here are the following recent approaches on the five abundant Indian coastal species carried on.

C. rotundata

In the early report, it has been said *C. rotundata* exhibit antimicrobial activity against *Bacillus cereus*, *Proteus vulgaris*, *Shigella*, *Escherichia coli*, *Shigella dysenteriae*, *Salmonella paratyphi*, *Staphylococcus aureus*, and *Pseudomonas fluorescens* [7].

The high-performance liquid chromatography analysis of ethanol extract of *C. rotundata* shows the presence of 19 phenolic compounds, namely, two compounds caffeic acid and α -coumaric acid are predominant. They exhibit high antioxidant activity [24]. Thus, *C. rotundata* can serve as a food supplement and can act as a nutraceuticals.

C. serrulata

In ancient history, *C. serrulata* were used as a tranquilizer for babies, as soothing helps during pregnancy and against a cough and malaria. The root extract of *C. serrulata* possess antimicrobial activity against five fish pathogen such as *Bacillus subtilis*, *Aeromonas hydrophila*, *Vibrio parahaemolyticus*, *Serratia* sp., and *Vibrio harveyi* [25]. They also exhibit antioxidant activity, ethyl acetate extract of *C. serrulata* leaves can kill HeLa cancer cells [26]. The active compound phenyl thioketone that exhibits antimicrobial activity was isolated from *C. serrulata* [27]. Silver nanoparticles synthesized from aqueous extract of *C. serrulata* possess higher free radical scavenging activity, the cytotoxicity assay on the cervical cancer (HeLa) and African green monkey kidney (Vero) cells on treatment with CSAE: 107.7 and 124.3 $\mu\text{g/ml}$ and CS-AgNPs: 34.5 and 61.24 $\mu\text{g/ml}$, respectively, showed good inhibition rate [28]. These findings highlight the fact that *C. serrulata* could be a potential source for developing potent drugs and further studies are needed.

Table 2: The phytochemical present in Cymodoceaceae seen in Indian coastal region

Seagrass	Phytochemical
<i>C. rotundata</i>	Tannins, saponins, proteins, resins, reducing sugar, acidic compounds, alkaloids, cardiac glycosides and terpenoids [7]
<i>C. serrulata</i>	Alkaloids, carboxylic acid, coumarins, flavonoids, phenols, saponins, xanthoprotein, protein, steroids, tannins and sugar [8]
<i>S. isoetifolium</i>	Resin, glycosides, reducing sugars, saponins, acidic compounds, cardiac glycosides, alkaloids [9]
<i>H. pinifolia</i>	Carbohydrates, alkaloids, cardiac glycosides, coumarins, flavonoids, phenols, saponin, tannin, terpenoids, xantho proteins [10]
<i>H. uninervis</i>	Flavonoids, alkaloids, steroids, phenols, tannin [11]

C. rotundata: *Cymodocea rotundata*, *C. serrulata*: *Cymodocea serrulata*, *S. isoetifolium*: *Syringodium isoetifolium*, *H. pinifolia*: *Halodule pinifolia*, *H. uninervis*: *Halodule uninervis*

Table 3: The common name and ethno use of Cymodoceaceae seen in Indian coastal region

Seagrass	Common name in Tamil Nadu	Ethno use
<i>C. serrulata</i>	Karumbu pasi, Peria korai pasi, Peria thazai pasi	Paste from leaves used to treat wounds
<i>C. rotundata</i>	Alai vaari, Kadal korai, Vellai thazahi pasi	They were eaten as food; their root has calorific value like potato
<i>H. pinifolia</i>	Nedung korai, Neetu korai, Arugampul pasi	Used as cattle feed and as manure
<i>H. uninervis</i>	Kothu korai, Panjipul pasi	In India it is used as a fertilizer
<i>S. isoetifolium</i>	Neer pasi, Oosi korai, Nool pasi	Fresh leaf juice consumed to ease acid reflux

C. rotundata: *Cymodocea rotundata*, *C. serrulata*: *Cymodocea serrulata*, *S. isoetifolium*: *Syringodium isoetifolium*, *H. pinifolia*: *Halodule pinifolia*, *H. uninervis*: *Halodule uninervis*

S. isoetifolium

This species is pickled and eaten by Philippines [29]. At recent study, acetone and methane extract of *S. isoetifolium* shows an antibacterial effect against 17 human pathogens and five fish pathogens [30].

H. pinifolia

H. pinifolia shows higher scavenging activity as it contains high phenolic compounds [9] fucoidan like sulfated polysaccharides were identified in *H. pinifolia* they exhibit good antioxidant activity and prolonged blood clotting time. Thus they can act as a good anticoagulant [31].

H. uninervis

The phenolic content of *H. uninervis* was analyzed to be 20.17 ppm, and content of tannin was 1,223 ppm. Antibacterial activity of an extract showed that seagrass can inhibit several types of Gram-negative bacteria (*A. hydrophila* and *V. harveyi*) and Gram-positive bacteria (*B. subtilis* and *Listeria monocytogenes*). The highest inhibitory activity present on the type of Gram-positive bacteria (*B. subtilis* and *L. monocytogenes*) is between 7 mm and 9 mm [32].

OTHER APPLICATIONS

Besides seagrass serving as a habitat and food source for various sea organisms, they were utilized by the coastal people in many ways. They were used as cattle feed by small-scale coastal farmers. They collect seagrass biomass accumulated at the sea shore and use it as green manure to garden. *C. serrulata* were used by local fishing market as insulation during the hot summer to keep stored ice and fish cool. Some of the seagrass has more fiber content and decay very slowly they were used to make mattress and padding material. As they absorb nutrients from coastal run-off and stabilize sediment helping to keep the water clear. Since they absorb metals, they were used as a bioindicator to check pollution of the ocean. Few species of seagrass have aesthetic and spiritual utility among coastal cultures.

PERSPECTIVE

Each species of seagrass Cymodoceaceae is unique in nature. The higher calorific value of seagrass which makes giant marine mammals such as sea cows, sea dungeons, and green turtles to graze them may be responsible for their long life span. The presence of phenolic compounds, alkaloids, and sulfated polysaccharides makes them a reservoir of potentially valuable and recoverable bioactive substance. Thus, these interesting factors of seagrass create a need for further research to treasure the seagrass metabolite content which may have economic importance.

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