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Research Article

# IDENTIFICATION OF BIOACTIVE COMPOUNDS IN *CYMODOCEA SERRULATA*-A SEAGRASS BY GAS CHROMATOGRAPHY–MASS SPECTROSCOPY

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**ABSTRACT**

**Objective:** The objective of this study was to identify the lead phytocompounds present in the ethanol extract of the seagrass *Cymodocea serrulata* by gas chromatography–mass spectrometry (GCMS).

**Methods:** 1 kg of *C. serrulata* whole seagrass powder was subjected to extraction on polarity basis using five solvent such as hexane, chloroform, ethyl acetate, ethanol, and water. Since ethanol extract showed a maximum antioxidant property, its phytochemicals were investigated using GCMS technique. The phytocompounds identified through GC were interpreted with mass spectra national institute standard and technology library.

**Result:** The GCMS analysis of ethanol extract of *C. serrulata* identified peaks of six different compounds they are hexahydrofarnesyl acetone (7.70%), hexadecanoic acid, methyl ester (4.11%), tetradecanoic acid (62.89%), pentadecanoic acid(62.89%), cholesta4, 6dien3ol (5.88%), and stigmasterol (19.42%).

**Conclusion:** The GCMS study of *C. serrulata* ethanol extract unveils the presence of bioactive compounds that have a pharmacological and nutraceutical values.

**Keywords:** Gas chromatography–mass spectrometry, *Cymodocea serrulata*, Seagrass, Palmitic acid, Phytocompounds.

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## INTRODUCTION

Our ancestor lived a life with a quote "food as a medicine." In mid-generation, people forgot about traditional foods, especially herbs come to know about various new diseases that are epidemic to humankind. In past decades, attention was turned toward the ethnobotanical use of our traditional herbs; it is mainly because of an outbreak of multiple resistance of pathogen against antibiotics. To search a novel and potent therapeutic agent, it is directed to look into the marine source, which yet has to be explored. Recently, there are many biologically active compounds such as fucoidans, phlorotannins, pigments, and phycocolloids were discovered from marine sources [1]. When we look into marine habitats, marine angiosperms are unique, they occupy the bottom line of food chain serve as main nutrients for the ocean higher organisms. Seagrass is marine angiosperm that grows and completes their lifecycle submerged under the ocean. Their structural organization and phytochemical composition are in such a way to adapt and protect themselves from salinity, wave strength, microorganism, epiphytes, and predation [2]. In folk medicine, *Cymodocea serrulata*, a seagrass commonly known as karumbu passi has been used as a food and also as a medicine by coastal region people and by fishermen while traveling in the sea [3]. It is used as a tranquilizer for babies as soothing helps during pregnancy and against cough and malaria. *C. serrulata* is seen abundant in South Indian coastal region. Although there is a report on the antibacterial, antioxidant, and anti-inflammatory property of *C. serrulata,* there are no evident details on phytocompounds present in it [4]. In the present study, the ethanol extract of *C. serrulata* was subjected to GC and mass spectroscopy analysis to elucidate the phytocompounds present behind their bioactivity.

## METHODS

### Collection of seagrass

The fresh seagrass *C. serrulata* was collected from Thirupalaikudi, Ramanathapuram district, coastal region during June by skilled divers. It has been identified and authenticated by Dr. N.kaliaperumal, Former Principal Scientist, CMFRI (ICAR, Govt. of India). The collected seagrass was washed thoroughly and shade dried. Then, the dried *C. serrulata* was powdered and preserved in an airtight container.

### Extraction

150 g of dried seagrass powder was soaked in 1:2 ratio in each solvent for 3 days successively in increasing polarity order through hexane, chloroform, ethyl acetate, ethanol, and aqueous at room temperature. The extracts were collected and filtered through Whatman filter paper and shade dried. As per the previous study, ethanol extract of *C. serrulata* shows an efficient antioxidant activity; thus, the ethanol extract of *C. serrulata* was subjected to gas chromatography–mass spectrometry (GCMS) analysis.

### GCMS

The phytocompounds present in ethanol extract of *C. serrulata* was identified using GC SHIMADZU QP2010 equipment. In this instrument, gas chromatogram was integrated with a mass spectrometer in such a way that the mass analyzer and detector were directly connected to the capillary column (RXI-17sil MS 30 m×0.25 mm×0.25 µ). While detection, electron ionization energy of 70eV was utilized and helium gas of 99.999% pure was used as a carrier gas at a constant flow rate of 1.5 ml/min. The sample was injected at 2 µl injection volume, the injector temperature and ion source temperature was set at 200°C. At 70 eV, mass spectra were carried out with a scan interval of 0.5 s at a 40–1000 m/z scan range. The total GC running time was 35 min. By comparing the average peak area to the total area, the relative percentage amount of each component was calculated. GCMS solution version 2.6 software was used for detection.

### Identification of compound

The phytocompounds were identified by comparing the spectrum of know component to the spectrum of the unknown component.

The software used will identify the compound by using similarity search, similarity search with index, and index search methods. The interpretation of mass spectrum was done with libraries such as the National Institute Standard and Technique, which has more than 62,000 patterns, Wiley, drug library, FFNSC library (flavor and fragrance). The name, molecular weight, molecular formula, and structure of the compound were determined.

## RESULT

The GCMS analysis of ethanol extract of the seagrass *C. serrulata* identified about nine bioactive components. Fig. 1 represents the chromatogram of different phytocompounds present in the ethanol extract of *C. serrulata.* Their principles of retention time, molecular weight, molecular formula, and peak area are given in Table 1.

The bioactive components identified are hexahydrofarnesyl acetone, hexadecanoic acid methyl ester, n-hexadecanoic acid, tetradecanoic acid, pentadecanoic acid, cholesta4,6dien3ol, and stigmasterol. At single retention time 18.86, there are three major components palmitic acid, myristic acid, and pentadecanoic acid were identified. They have the highest peak value of 62.89%. The derivative of stigmasterol, stigmasta5, and 22-dien3ol acetate was identified at the high peak level of 19.42%. Palmitic acid esters were identified to be at 14.11%. The two components hexahydrofarnesyl acetone and 2undecanone 6,10dimethyl were identified to be at the peak value of 7.70%. The other two components are stigmasterol, and Cholesta-4,6-dien 3ol were at 5.88%. The structure of these compounds identified through GCMS is given in Table 2.

## DISCUSSION

The GCMS analysis of ethanol extract of *C. serrulata* reveals the presence of phytocompounds that are biologically active. According to the chromatogram obtained by GCMS ethanol extract of *C. serrulata* consists of palmitic acid, myristic acid, and pentadecanoic acid as a major component. They may be produced by the plant defense itself from stress as secondary metabolites. These phytoprotectants proved to possess pharmacological activity acts in similar away as synthetic drugs [12]. The palmitic acid reported possessing anticancer activity, antimicrobial, and nematicide activity. The palmitic acid increases the number of probiotic bacteria in the gut; thus, they involved in the development of intestine [13]. It is required in the biosynthesis of lung lecithin, which is related to fetal maturation as well as it has been reported that presence of palmitic acid in the Nigerian meal can partly be related to the low incidence of respiratory disease [14]. Palmitic acid reported inhibiting human hepatoma cell growth in a dose-dependent and time-dependent manner. Thus, they possess anticancer activity [15].

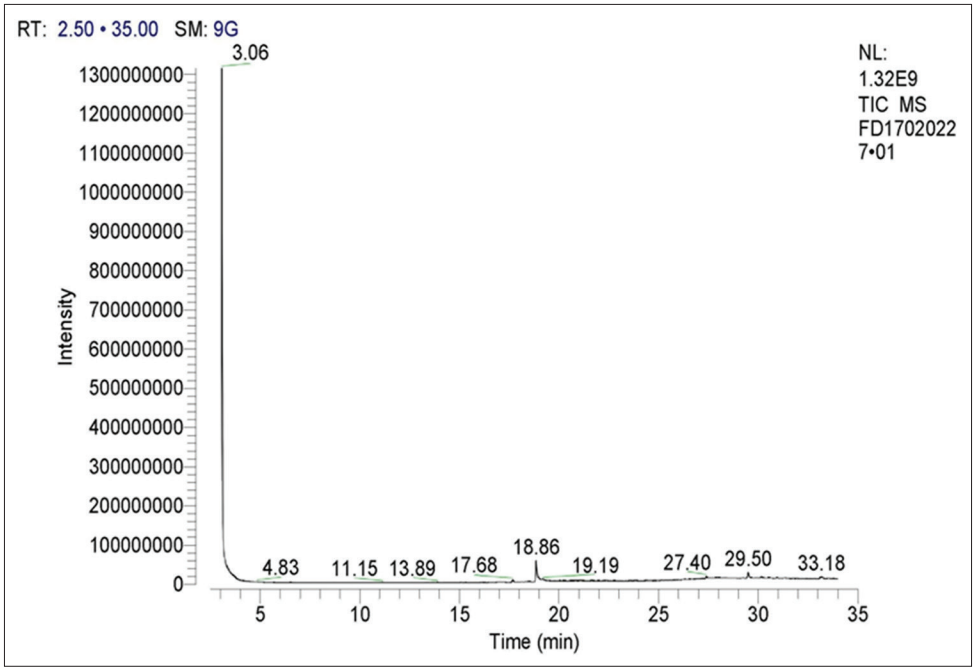


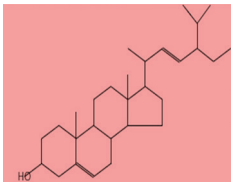
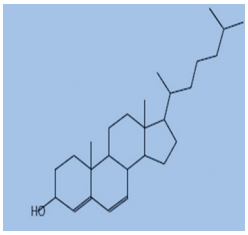
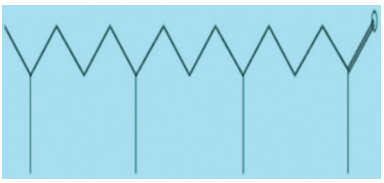
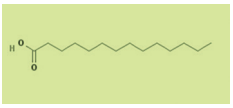
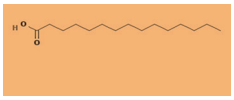

**Fig. 1: Gas chromatography–mass spectrometry chromatogram of ethanol extract of *Cymodocea serrulata***

**Table 1: Phytocompounds identified in ethanol extract of *C. serrulata* by GCMS**

| S.NO. | Retention time | Compound name | Molecular formula | Molecular weight | Peak area % |
|---|---|---|---|---|---|
| 1. | 17.68 | 2Pentadecanone, 6,10,14trimethyl (Hexahydrofarnesyl acetone) | C18H36O | 268 | 7.70 |
| | | 2Undecanone, 6,10dimethyl | C13H26O | 198 | 7.70 |
| 2. | 18.52 | Hexadecanoic acid, methyl ester (Palmitic acid) | C17H34O2 | 270 | 14.11 |
| 3. | 18.86 | nHexadecanoic acid | C16H32O2 | 256 | 62.89 |
| | | Tetradecanoic acid (Myristic acid) | C14H28O2 | 228 | 62.89 |
| | | Pentadecanoic acid | C15H30O2 | 242 | 62.89 |
| 4. | 27.40 | Stigmasterol | C29H48O | 412 | 5.88 |
| | | Cholesta4,6dien3ol | C27H44O | 384 | 5.88 |
| 5. | 29.50 | Stigmasta5,22dien3ol, acetate, | C31H50O2 | 454 | 19.42 |

GCMS: Gas chromatography–mass spectrometry, *C. serrulata: Cymodocea serrulata*

**Table 2: Biological activity of identified compounds present in the ethanol extract of *C. serrulata***

| S. NO. | Compound name | Structure | Nature of compound | Biological activity |
|---|---|---|---|---|
| 1 | Hexadecanoic acid (Palmitic acid) |  | Fatty acid | Antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic flavor, hemolytic, and alpha-reductase inhibitor [5] |
| 2. | Stigmasta5,22dien3ol (Stigmasterol) |  | Steroids | Antiosteoarthritic, antihypercholesterolemic, cytotoxicity, antitumor, hypoglycaemic, antimutagenic, antioxidant, anti-inflammatory, and CNS effects [6]. |
| 3. | Cholesta4,6dien-3-ol, |  | Steroids | Wound healing property, skin protective property, and antioxidant activity [7]. |
| 4. | Hexahydrofarnesyl acetone |  | Essential oil | Antihypertensive activity, vasodilation effect toward cerebral, and basilar artery [8]. |
| 5. | Tetradecanoic acid (myristic acid) |  | Fatty acid | Antioxidant, cancer preventive, nematicide, hypercholesterolemic, and lubricant [9,10]. |
| 6. | Pentadecanoic acid |  | Fatty acid | Many odd length amino acids are derived from pentadecanoic acid. Act as a biological marker for the dietary milk intake [11]. |

*C. serrulata: Cymodocea serrulata*

The myristic acid (Tetradecanoic acid) is an essential fatty acid has the variety of application in the beauty industry as fragrance ingredients, opacifying agent, surfactant, cleansing agent, and emulsifier. Mainly, it has been applied as a lubricant since it has a high rate of absorption by the skin. Myristic acid is a membrane stabilizer as they act as a lipid anchor for proteins [16]. It plays a role in energy storage as they increase LDL level. It has been reported that diabetic HDL- associated with myristic acid inhibits the stimulation of nitric oxide generation [17]. These findings have important implications regarding cardiovascular disease in diabetic patients.

The pentadecanoic acid is an odd chain saturated fatty acid with a 15-carbon backbone is of exogenous origin not synthesized by the animal. It has been reported that it is seen highly in adipose tissue of person who intakes frequently dairy and fish food; thus, they act as a biomarker. The pentadecanoic acid act as a substrate for the synthesis of odd-numbered, very long-chain fatty acids which are glycosphingolipids in the brain. It provides anaplerotic intermediates for the citric acid cycle by a convert to propionyl-CoA and further into succinyl-CoA. It removes excess of propionic acid from the circulation [18].

Stigmasterol is a phytosterol produced by various medicinal plants were found to be in a higher component in ethanol extract of *C. serrulata*. Stigmasterol is a precursor for the synthesis of progesterone [19]. Since they are the intermediate in the biosynthesis of Vitamin D3, they act as an antiosteoarthritic [20]. It is also an intermediate in androgen, estrogen, and corticoids biosynthesis. Stigmasterol can inhibit cholesterol biosynthesis by inhibiting sterol Δ24-reductase in human Caco-2 and HL-60 cell lines; thus, they are the suppressor of hepatic cholesterol. Stigmasterol shows decrease in hepatic lipid peroxidation and increase in the activities of catalase, superoxide dismutase, and glutathione; thus, they are the good antioxidant [21].

Hexahydrofarnesyl acetone is a terpene ketone reported to possess long-lasting antihypertensive activity in spontaneous hypertensive rat without altering the heart rate [22]. Some amount of cholesta-4,6-dien-3-ol, is seen ethanol extract of *C. serrulata* which can act as an antioxidant and also possess wound healing property. The major chemical component of ethanol extract of *C. serrulata* identified by GCMS is saturated fatty acids, which possess significant biological activity.

## CONCLUSION

The GCMS analysis of ethanol extract of *C. serrulata* revealed the presence of the compound that has some ecological significance. The finding of these chemical components justifies their use as a remedy for the various ailments traditionally by our ancestors. The possession

of antioxidant, anticancer, and anti-inflammatory property proved that *C. serrulata* can act as a nutraceutical and as ayurvedic medicine in this disease evolving world. Further studies aiming to evoke the uses of *C. serrulata* as the food and medicine are needed.

## AUTHOR'S CONTRIBUTION

Pushpa Bharathi.N has performed the experiment, collected the data, and drafted the manuscript. Jayalakshmi.M has made significant involvement in the interpretation of data and revising the manuscript. Amudha.P participated in the proofreading of the manuscript. Vanitha.V designed the study and manuscript.

## CONFLICT OF INTERESTS

There is no conflict of interest between authors.

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