

## Original article

## Seagrasses as potential source of fodder for livestock: Complete proximate and gas chromatography-mass spectrometry (GC-MS) analysis

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**Abstract**

The present study analysed the nutritional values of *Cymodocea serrulata* (R.Br.) Asch. & Magnus, *Syringodium isoetifolium* (Asch.) Dandy and *Enhalus acoroides* (L.f.) Royle by complete proximate analysis and determined the phytochemicals present in them by using Gas Chromatography-Mass Spectrometry (GC-MS) technique. The complete proximate analysis revealed a higher content of crude protein, crude fibre, calcium, phosphorus and energy in all the three grasses under study. However *E. acoroides* contained higher content of ether extract, lower level of acid insoluble ash and gross energy compared to the other two grasses. The GC-MS analysis showed the presence of decane, sucrose, tetradecanoic acid, deoxyspergualin, n-hexadecanoic acid, phytol, ricinoleic acid, docosyl isobutyl ether and squalene in *C. serrulata* and decane, sucrose, phytol, 9,12,15-octadecatrienoic acid, (Z,Z,Z)-Triethylene glycol monododecyl ether and Hexadecanoic acid and 2-hydroxy-1-(hydroxymethyl) ethyl ester in *S. isoetifolium*. Ethanol extract of *E. acoroides* revealed the existence of decane, epoxomicin, tetradecanoic acid, n-hexadecanoic acid, phytol, linoleic acid, 9,12,15-octadecatrienoic acid, (Z,Z,Z)-diethylene glycol monododecyl ether, hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester and 15,17,19,21-hexatriacontatetrayne in GC-MS study. All the three seagrasses under study were found to be the rich sources of crude protein, energy, minerals and phytochemicals of varied pharmacological properties which can enable them to be an eligible source of alternative nutritional supplements for ruminants in the areas adjacent to coastal region of Ramanathapuram district, where fodder scarcity exists.

**Key words:** *Cymodocea serrulata* (R.Br.) Asch. & Magnus, *Syringodium isoetifolium* (Asch.) Dandy, *Enhalus acoroides* (L.f.) Royle, complete proximate analysis, gas chromatography-mass spectrometry, phytochemicals

**1. Introduction**

Seagrasses are submerged type of flora, found in the marine ecosystem, adapted to grow well in the intertidal and midtidal zones of shallow and sheltered areas of sea waters, backwaters and lagoons. They are monocotyledonous plants not belonging to the true grass family *Poaceae*, but belong to four plant families, viz., *Posidoniaceae*, *Zosteraceae*, *Hydrocharitaceae*, or *Cymodoceaceae* which are successfully adapted to the marine ecosystem (Ravikumar *et al.*, 2010). In Tamil Nadu, seagrasses are abundantly found in the Gulf of Mannar Biosphere Reserve (Mannar coast and Palk Bay). The Gulf of Mannar coast (area between Pamban and Tuticorin) had predominant species like *Halodule uninervis*, *Thalassia hemprichii* and *Cymodocea serrulata*. The Palk Bay (area between

Pamban and Athirampattinam) had a stretch of seagrass cover with dominant species like *Thalassia hemprichii*, *Syringodium isoetifolium* and *Cymodocea serrulata* (Ravikumar *et al.*, 2005). A total of 13 species including 6 genera of seagrasses present in the Mannar Biosphere Reserve. *Enhalus acoroides*, *Halophila ovalis*, *Halophila ovata*, *Halophila beccari*, *Halophila stipulacea*, *Thalassia hemprichii*, *Cymodocea serrulata*, *Cymodocea rotundata*, *Halodule uninervis*, *Syringodium isoetifolium* are some of the seagrasses available in this biosphere reserve. *Thalassia* and *Syringodium* are dominant in the areas of coral reefs and coral rubble, whereas others are distributed in muddy and sandy soils (Envis, 2015).

*C. serrulata*, a seagrass occurs extensively in most of the islands of Gulf of Mannar and forms a significant browsing ground for the endangered mammal, *Dugong dugon* (ENVIS, 2015). It is commonly known as "kadal karumbu" and can be used as food and medicine by fisherman and is utilised by animals as a type of grazing fodder, when fodder scarcity arises (Bharathi *et al.*, 2016). *S. isoetifolium*, a sea grass belongs to the family *Cymodoceaceae*, apparently seen in the Mannar Biosphere Reserve and contains

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large amount of valuable phytochemicals like saponins, flavonoids and alkaloids and are known for its medicinal uses (Albert *et al.*, 2015). *E. acoroides* belonging to the monotypic marine genus *Enhalus* in the family *Hydrocharitaceae* which contain sterol, lignin content and fatty acid components which can be used as a fodder supplement for animals (Gillan *et al.*, 1984).

Seagrasses are important biomass of marine ecosystem and produce greater amount of organic material, even supply basic source of energy in a complicated food web. In India, seagrasses are widely used as medicine, food, fertilizers and livestock feeds (Newmaster *et al.*, 2011; Athiperumalsami *et al.*, 2008; Pradheeba *et al.*, 2011). The crude extracts of seagrasses from the Gulf of Mannar had strong antibacterial (Ragupathi Raja Kannan *et al.*, 2010a; Arumugam *et al.*, 2010) and antioxidant properties (Ragupathi Raja Kannan *et al.*, 2010b; Ragupathi Raja Kannan *et al.*, 2012b). GC-MS analysis is one of ideal chromatographic techniques to find out the phytoconstituents in seagrasses. The phytochemicals are the most essential non-nutritive components secreted by plants and are also known as secondary metabolites which are not utilized by the plants, but used for protective or disease preventive purposes to the human beings (Das and Gezici, 2018). These secondary metabolites are relatively produced in low quantities in plants and have significant economic and medicinal values (Dang, 2018).

The interesting information from the earlier reporters motivated us to undergo this basic level study while observing the consumption of certain ashore plants by the local ruminants in some coastal areas of Ramanathapuram district, Tamil Nadu, India, which is a drought prone area with poor people depending mostly on livestock rearing for their livelihood. The baseline information generated through this study may pave way for the economical usage of these grasses or the product of these grasses as livestock feed/feed supplements.

## 2. Materials and Methods

### 2.1 Materials

#### 2.1.1 Collection of plants

The ashore plants found to be consumed by the local goats were collected from the coastal areas near Seeniappa Dharka and Devipattinam of Ramanathapuram district during May 2019 (summer season).

### 2.2 Methods

#### 2.2.1 Identification

The species identification of the plants was done at ICAR-Central Marine Fisheries Research Institute, Mandapam, Ramanathapuram district by gross and microscopic methods and a voucher specimen for *Cymodocea serrulata*, *Syringodium isoetifolium* and *Enhalus acoroides* were deposited at the centre with identification number VUTRC/RMD/CS 01, VUTRC/RMD/SI 02 and VUTRC/RMD/EA 03, respectively.

#### 2.2.2 Preparation of plants for investigation

The collected samples of three different plants were repeatedly washed for 5-6 times in freshwater to remove the traces of impurities. The leaves were shade dried, mechanically pulverised.

#### 2.2.3 Preparation of extracts

About 150 g of grinded powder was initially soaked into 1:2 ratio of ethanol for 3 days with mild shaking. After 3 days, the ethanol was decanted, and the collected extract was stored at room temperature in a dark place, and the ethanol solvent was evaporated.

Finally, solid material was collected and stored at 4°C until used (Pushpabharathi *et al.*, 2018).

### 2.2.4 Complete proximate analysis

The complete proximate analysis was carried out as per the method of AOAC (2000) to determine the presence of nutritional fractions in the grinded material of the collected plants.

### 2.2.5 GC-MS analysis

GC-MS analysis was carried out at the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore using GC-MS-5975C (AGILENT) under the following conditions as per Gomathi *et al.* (2015). DB-5ms Agilent (30.0 m × 0.25 mm × 0.25 μm) was used. Using helium as carrier gas (99.9995% purity) at a constant flow rate of 1.51 ml/min and an injection volume of 2 μl was employed in a split mode. The injector temperature was maintained at 240°C, and the column temperature was programmed to 70°C (isothermal for 2 min) with increasing temperature of 10°C/min to 300°C (isothermal for 9 min). 200°C for ion source temperature and 240°C for interface temperature were maintained. The mass spectra were obtained through ionization energy of 70 eV in the EI mode. Total 30 min need to run GC-MS. The phytochemicals were identified by comparison of mass spectra with the database of National Institute of Standard and Technology (NIST-11) which has 62,000 patterns. The compound name, retention time and the structure were determined. Each components percentage of area was calculated by comparing its average peak area to the total areas. Prediction of biological activities of each compound was done based on as per Duke (2019).

## 3. Results

The freshly collected fully matured plants were identified as *C. serrulata*, *S. isoetifolium* and *E. acoroides*. The grasses collected from Seeniappa Dharka were *C. serrulata* and *S. isoetifolium* and the grass from Devipattinam area was *E. acoroides*. The complete proximate analysis revealed a higher content of crude protein, crude fibre, calcium, phosphorus and energy in all the three grasses under study. However, *E. acoroides* contained higher content of ether extract, lower level of acid insoluble ash and gross energy compared to the other two grasses (Table 1).

The GC-MS analysis of ethanol extract of *C. serrulata*, *S. isoetifolium* and *E. acoroides* revealed the presence of phytochemicals that are biologically active. GC-MS chromatogram of *C. serrulata* showed 39 phytochemicals (Figure 1), *S. isoetifolium* showed 38 phytochemicals (Figure 2) and *E. acoroides* showed 36 phytochemicals (Figure 3). The retention time, compound name, peak area, nature of compound and biological activity of *C. serrulata*, *S. isoetifolium* and *E. acoroides* are tabulated (Table 2 to 4) as Figure legends with the biological activity of phytochemicals. The notable phytoconstituents seen in *C. serrulata* were docosyl isobutyl ether (10.94%), sucrose (9.22%), ricinoleic acid (5.50%), n-hexadecanoic acid (4.56%) and squalene (3.79%). The significant phytoconstituents seen in *S. isoetifolium* were n-hexadecanoic acid (12.90%), sucrose (8.06%), 9,12,15-octadecatrienoic acid, (Z,Z,Z)- (3.22%) and triethylene glycol monododecyl ether (2.80%). The phytochemicals seen in *E. acoroides* were n-hexadecanoic acid (15.40%), diethylene glycol monododecyl ether (12.85%), 9,12,15-octadecatrienoic acid (9.15%) and hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (3.74%).

**Table 1:** Proximate analysis of *C. serrulata*, *S. isoetifolium* and *E. acoroides*

S.No	Test	<i>Cymodocea serrulata</i>	<i>Syringodium isoetifolium</i>	<i>Enhalus acoroides</i>
1.	Moisture	10.35%	11.06%	13.42%
2.	Crude Protein	9.18%	9.08%	8.73%
3.	Crude Fibre	17.50%	17.46%	13.07%
4.	Ether Extract	1.67%	1.56%	2.07%
5.	Total Ash	21.74%	24.66%	24.45%
6.	Acid Insoluble Ash (Sand & Silica)	4.94%	4.31%	2.02%
7.	Calcium	2.00%	1.59%	1.70%
8.	Phosphorus	0.28%	0.25%	0.19%
9.	Salt	9.52%	12.60%	15.68%
10.	Gross Energy	2959 kcal/kg	2808 kcal/kg	2742 kcal/kg

**Table 2:** Phytochemicals identified in the ethanol extract of *C. serrulata* using GC-MS analysis

Retention time	Compound name	Peak area %	Nature of compound	Biological activity*
3.26	Decane	0.80	Alkane	Antibacterial activity
9.50	Sucrose	9.22	Disaccharides	Preservative
15.28	Tetradecanoic acid	0.94	Fatty acid derivative	Antioxidant, cancer preventive, nematocide, hypercholesterolemic, and lubricant
17.93	Deoxyspergualin	1.03	Polyphenols	Anti-angiogenic action, cytoprotection, Immunomodulation
19.30	n-Hexadecanoic acid	4.56	Saturated fatty acid derivative	Antioxidant, hypocholesterolemic, nematocide, pesticide, antiandrogenic, hemolytic, and alpha reductase inhibitor
22.01	Phytol	0.96	Diterpene alcohol	Antimicrobial, anti-inflammatory, anticancer and diuretic
25.71	Ricinoleic acid	5.50	Unsaturated fatty acid derivative	Antibacterial and antifungal
27.25	Docosyl isobutyl ether	10.94	Ether derivatives	Disinfectant, lubricants
29.20	Squalene	3.79	Triterpene	Antibacterial, antioxidant, antitumor, cancer preventive, immune stimulant, chemo preventive, lipoxygenase inhibitor, pesticide

\*Duke. Phytochemical and ethnobotanical databases

**Table 3:** Phytochemicals identified in the ethanol extract of *S. isoetifolium* using GC-MS analysis

Retention time	Compound name	Peak area %	Nature of compound	Biological activity*
3.27	Decane	2.13	Alkane	Antibacterial activity
9.57	Sucrose	8.06	Disaccharide	Preservative
19.34	n-Hexadecanoic acid	12.90	Fatty acid derivative	Antioxidant, hypocholesterolemic, nematocide, antiandrogenic hemolytic and alpha reductase inhibitor
22.03	Phytol	1.56	Diterpene alcohol	Antimicrobial, anti-inflammatory, anticancer and diuretic
22.42	Linoelaidic acid	1.24	Isomer of linoleic acid and diabetes	Anti-cancer, atherosclerosis, obesity, immuno-modulator
22.52	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	3.22	Fatty acid derivative	Hypocholesterolemic, nematocide, antiarthritic, hepatoprotective, antiandrogenic, 5-Alpha reductase inhibitor, antihistaminic, anticoronary, insectifuge, antieczemic, antiacne
25.08	Triethylene glycol monododecyl ether	2.80	Ether derivatives	Disinfectant, lubricant
28.66	Hexadecanoic acid, 2-hydroxy-1-(hydroxyl methyl) ethyl ester	0.90	Fatty acid derivative	Hemolytic, pesticide, flavouring agent, antioxidant

\*Duke. Phytochemical and ethnobotanical databases

**Table 4:** Phytochemicals identified in the ethanol extract of *E. acoroides* using GC-MS analysis

Retention time	Compound name	Peak area %	Nature of compound	Biological activity*
3.27	Decane	1.53	Alkane	Antibacterial activity
5.75	Epoxomicin	1.48	Proteasome inhibitor	Anti-inflammatory, parkinson disease
15.38	Tetradecanoic acid	1.47	Fatty acid derivative	Antioxidant, cancer preventive, nematocide and hypercholesterolemic
19.33	n-Hexadecanoic acid	15.40	Fatty acid derivative	Antioxidant, hypocholesterolemic, nematocide, antiandrogenic, hemolytic and alpha reductase inhibitor
22.01	Phytol	1.49	Diterpene alcohol	Antimicrobial, anti-inflammatory, anticancer and diuretic
22.42	Linoelaidic acid	1.96	Isomer of linoleic acid and diabetes	Anti-cancer, atherosclerosis, obesity, immuno-modulator
22.51	9,12,15-Octadecatrienoic acid	9.15	Fatty acid derivative	Hypocholesterolemic, nematocide, antiarthritic, hepatoprotective, antiandrogenic, 5-Alpha reductase inhibitor, antihistaminic, antiacne anticoronary and Antieczemic
27.27	Diethylene glycol monododecyl ether	12.85	Ether derivatives	Disinfectant, lubricant
28.65	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	3.74	Fatty acid derivatives	Lubricants, emollients
28.78	15,17,19,21-Hexatriacontatetrayne	0.74	Acyclic alkanes	Antioxidants

\*Duke. Phytochemical and ethnobotanical databases

#### 4. Discussion

The present study revealed the presence of higher level of crude protein, highest (9.18%) in *C. serrulata* and lowest (8.73%) in *E. acoroides*, crude fibre, highest (17.50%) in *C. serrulata* and lowest (13.07%) in *E. acoroides*, total ash, 21.74% in *C. serrulata*, 24.66% in *S. isoetifolium* and 24.45% in *E. acoroides* and calcium 2.00% in *C. serrulata*, 1.59% in *S. isoetifolium* and 1.70% in *E. acoroides*. Ragupathi Raja Kannan *et al.* (2013) reported the nutritional composition like ash, protein, carbohydrate, lipids, fibre, ash, *etc.* in six sea grasses including *E. acoroides*, *S. isoetifolium* and *C. serrulata* and stated that protein content ranged from 5.61 to 14.40% with maximum in *C. rotundata* and least in *E. acoroides*. Similarly, the crude fibre content was higher in *C. serrulata* and least in *E. acoroides*, was in agreement with the study of Ragupathi Raja Kannan *et al.* (2013). Kanwar *et al.* (1997) pointed out that the WHO has recommended an intake of 22-23 g of fibre every 1000 kcal of diet. This quantum of fibre is very much available in these 3 seagrasses, studied especially in *C. serrulata* and *S. isoetifolium* with highest sucrose content ascertained by GC-MS analysis during this study. The sucrose might be reason for the sweeter taste and palatability of the grass, especially during summer seasons (Drew, 1983). The optimum amount of quality fibre available could be well utilised by the ruminants for their better digestibility without constipation.

The GC-MS analysis showed the presence of fatty acid compounds like Tetradecanoic acid, Ricinoleic acid, n-hexadecanoic acid, terpenoids like phytol and squalene, ether derivatives like docosyl isobutyl ether, phenolic derivatives like deoxyspergualin, alkane group like decane and disaccharides like sucrose in *C. serrulata* and decane, sucrose, phytol, hexadecanoic acid, 9,12,15-octadecatrienoic acid (Z,Z,Z)-(fatty acid derivative), Triethylene glycol monododecyl

ether and 2-hydroxy-1-(hydroxymethyl) ethyl ester in *S. isoetifolium*. Ethanol extract of *E. acoroides* revealed the existence of decane, epoxomicin (peptide), tetradecanoic acid, 9,12,15-octadecatrienoic acid, (Z,Z,Z)-n-hexadecanoic acid, linoelaidic acid, diethylene glycol monododecyl ether, hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, phytol and 15,17,19,21-hexatriacontatetrayne (alkane) in GC-MS study. Ravikumar *et al.*, (1993) had reported that *C. serrulata* contained rich bioactive compounds like alkaloids, carboxylic acid, coumarins, flavonoids, phenols, saponins, xanthoprotein, protein, steroids, tannins and sugar. Mani *et al.*, (2012) reported that *S. isoetifolium* contained rich amount of phytoconstituents like resin, glycosides, reducing sugars, saponins, acidic compounds, cardiac glycosides and alkaloids. Vergeer *et al.*, (1995) stated that sea grasses underwent various stress factors and explicit ether derivatives as bioactive compounds which could be used as a disinfectant, emollient and lubricants in skin. The stress was found to be augmented by various vital factors like competitors, predators and continuous grazing conditions. Besides ether, other bioactive components like phenolic acids, sulfated phenolic acids, flavones, condensed tannins, flavonoids and also lignins were elucidated because of stress condition. The increased ether derivatives and polyphenols in this study could also be an indicator of such stresses caused to the grasses. The fatty acid, alcohol and ether derivatives were the common phytochemicals found in all the 3 seagrasses studied. However, deoxyspergualin a polyphenol derivative and phytol and squalene the terpenoid derivatives are seen only in *C. serrulata* additional to the phytochemicals studied. This study revealed that *C. serrulata*, *S. isoetifolium* and *E. acoroides* had both medicinal and fodder values and hence could be commented as plants of phytopharmaceutical importance.

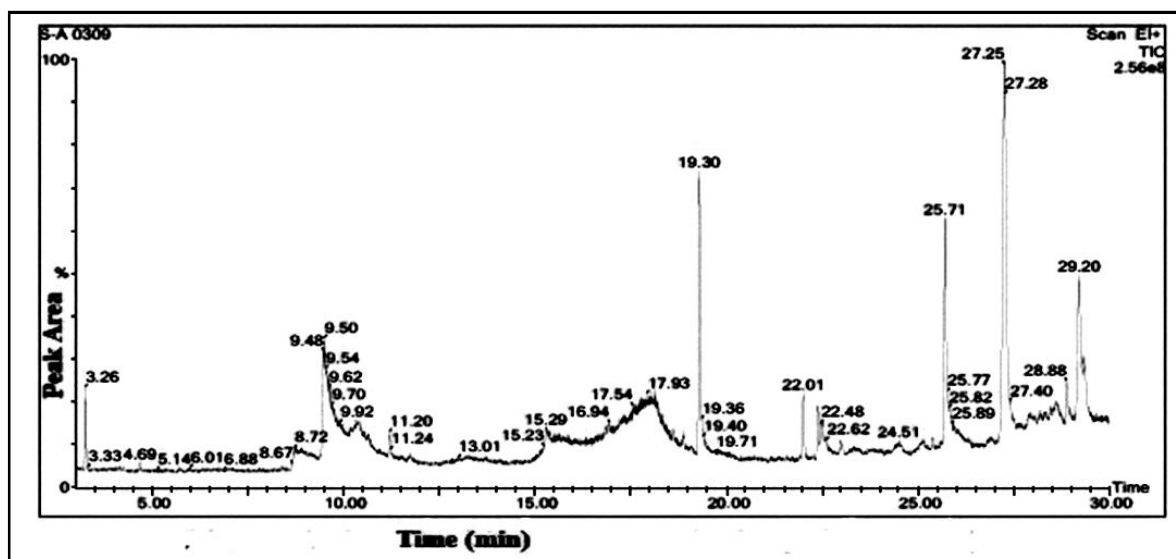


Figure 1: Gas chromatography-mass spectrometry chromatogram of ethanol extract of *C. serrulata*.

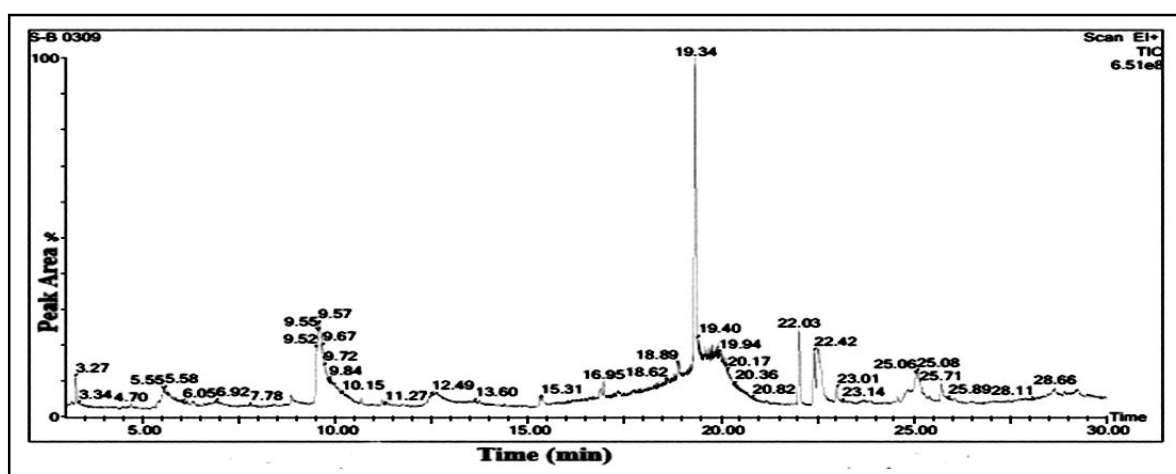


Figure 2: Gas chromatography-mass spectrometry chromatogram of ethanol extract of *S. isoetifolium*.

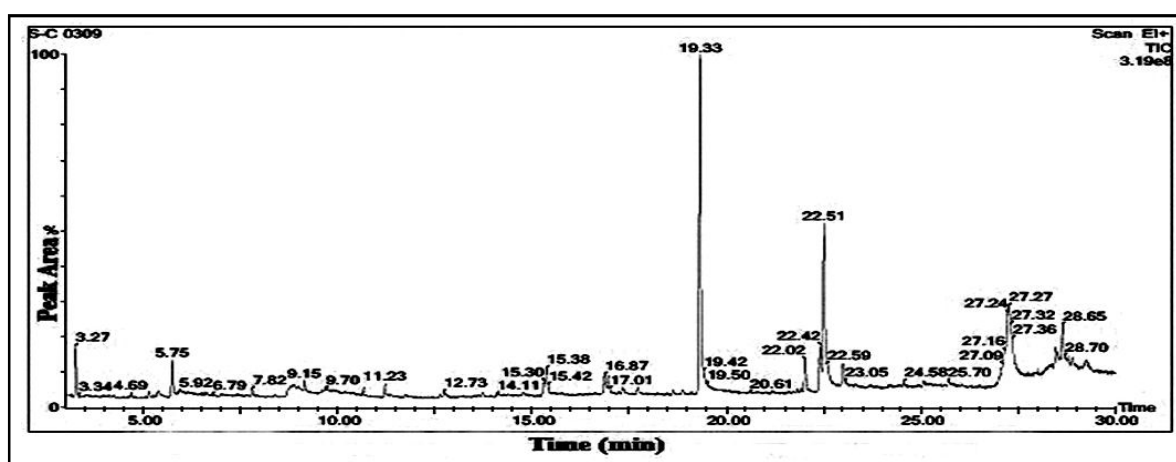


Figure 3: Gas chromatography-mass spectrometry chromatogram of ethanol extract of *E. acoroides*.

## 5. Conclusion

These species of seagrasses are unique in nature and are the feeding source for marine mammals such as sea cows and green turtles. However, the suitability of these seagrasses as a fodder alternative/feed supplement for grazing domestic animals has to be ascertained. In such instances, ways may be thought of to collect these grasses without disturbing the ecological conditions of the sea or to artificially cultivate such grasses in the coastal areas or in certain areas of Ramanathapuram district of Tamil Nadu, India where the ground water is mostly salty in nature. Utmost care should be taken not to disturb the natural biosphere of these areas.

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## Conflict of interest

The authors declare that there are no conflicts of interest in the course of conducting the research. Both the authors had final decision regarding the manuscript and decision to submit the findings for publication.

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