ASIAN J NAT PROD BIOCHEM
Volume 22, Number 2, December 2024
Pages: 74-88

E-ISSN: 2580-2550
DOI: 10.13057/biofar/f220204

Bioactive compounds of seven seagrass species from the Western Indian Ocean identified by Gas Chromatography-Mass Spectrometry

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Manuscript received: 7 February 2024. Revision accepted: 12 August 2024.

Abstract. *Maganga PY, Mbusi LD, Hamisi MI, Mgina CA, Lyimo TJ. 2024. Bioactive compounds of seven seagrass species from the Western Indian Ocean identified by Gas Chromatography-Mass Spectrometry. Asian J Nat Prod Biochem 22: 74-88.* Seven seagrass species from the coast of Tanzania, namely *Cymodocea serrulata, Halodule uninervis, Enhalus acoroides, Cymodocea rotundata, Syringodium isoetifolium, Thalassia hemprichii,* and *Thalassodendron ciliatum,* were previously reported to have antibacterial activity against pathogenic microorganisms. This study presents the results of quantitative analysis of the phytocompound composition of hexane extracts of these seagrass species using Gas Chromatography-Mass Spectrometry technique. Overall, 24 known biologically active phytocompounds were revealed, with commonly found compounds being steroids, namely stigmasta-5,22-dien-3-ol acetate and 3*β* sitosterol acetate. On the other hand, 3*β*-cholest-5-en-3-ol tetradecanoate was found only in *C. rotundata* and *T. ciliatum.* Hydrocarbons (1-nonadecene and tetracosane) and a diterpene (phytol acetate) were found only in *H. uninervis* and *T. ciliatum.* In addition, pentadecanal and n-hexadecanoic acid were found in *C. rotundata* and *T. hemprichii,* respectively, while heneicosane and hexadecanoic acid methyl ester were found in *T. ciliatum* only. Moreover, *T. ciliatum* was found to contain more compounds than other seagrass species. This study reports for the first time the occurrence of fourteen compounds from seagrass species. All seven seagrass species are rich sources of phytocompounds with various pharmacological properties, such as antimicrobial, antioxidant, and antiinflammatory activities.

Keywords: Biological activities, Gas Chromatography-Mass Spectrometry, hexane extracts, phytocompounds, seagrass roots and leaves

INTRODUCTION

Seagrasses are plants that live submerged in shallow water areas of temperate, subtropical, and tropical seas **(**Kannan and Thangaradjou 2005; Togashi et al. 2007). They are monocotyledonous plants belonging to four plant families, Posidoniaceae, Zosteraceae, Hydrocharitaceae, and Cymodoceaceae, which have successfully adapted to the marine ecosystems (Ravikumar et al. 2010). There are 60 seagrass species belonging to 13 genera distributed all over the world (Gacia et al. 2003). Seven genera (*Cymodocea*, *Enhalus*, *Halodule*, *Halophila*, *Syringodium*, *Thalassia,* and *Thalassodendron*) are tropical. The remaining six genera (*Amphibolis*, *Heterzostera*, *Phyllospadi*, *Posidonia*, *Pseudalthenia,* and *Zostera*) are largely confined to temperate waters (Ravikumar et al. 2010). The coastal zones of the Western Indian Ocean (WIO) region harbor 14 known species that comprise about 25% of all seagrasses worldwide (Amone-Mabuto et al. 2017). The species occurring in the WIO region are *Enhalus acoroides* (U.) Royle, *Halophila minor* (Zoll.) den Hartog, *H. ovalis* (R. Br.) Hook. f., *H. stipulacee* (Forsk.) Aschers., *Thalassia hemprichii* (Ehrenberg) Asherson, *Zostera capensis* Setchell, *Cymodocea rotundata* Ehrenb. et Hempr. ex Aschers., *C. serrulata* (R. Br.) Aschers. et Magnus, *Halodule uninervis* (Forsk.) Aschers. in Bossier, *H. wrightii* Ascherson, *Syringodium isoetifolium* (Ascherson) Dandy, *Thalassodendron ciliatum* (Forsk.) den Hartog., *T. leptocaule* MC Duarte, Bandeira & Romeiras, and *Ruppia maritina* L. (Gullström et al. 2002; Duarte et al. 2012). The distribution of seagrasses depends on a series of physical factors such as temperature, turbidity, salinity, substrate type, and light availability, where the presence or absence of species depends on their unique adaptation and ability to tolerate those environmental factors (Ha et al. 2019). Twelve seagrass species have been encountered in Tanzania, with the most extensive meadows found on beaches or cliffs and adjacent fringing reefs (Ochieng and Eftemeijer 2003; Hamisi et al. 2004).

Seagrasses are rich sources of secondary metabolites that are believed to be a defense mechanism for these plants, some of which are potential antimicrobial compounds that reduce or control microbial growth (Kannan et al. 2010a; Sangeetha and Asokan 2016; Hamisi et al. 2023). Therefore, recognizing seagrass's potential, several endeavors have been made globally from which various secondary metabolites with pharmacological properties have been extracted and tested. Gono et al. (2022) reported the antiviral, antibacterial, antifungal, antiprotozoal, antifertility, and pharmacological properties of seagrass extracts. Furthermore, seagrass extracts of *H. pinifolia*, *H. ovalis*, and *T. hemprichii* from the southeast coast of India were reported to have antiviral activity (Premanathan et al. 1992). In addition, Kannan et al. (2010a) reported the

antibacterial activity of seagrasses *H. stipulacea*, *C. serrulata*, and *H. pinifolia* from the Mandapam coast, India. Moreover, Saranya et al. (2017) reported that the seagrasses *H. ovalis* and *T. hemprichii* collected from the Keelakarai Coast, Ramnad, Tamil Nadu, have interesting biochemical and bioactive potentials.

In Tanzania, seagrasses have been used as traditional medicine. These include *E. acoroides*, whose roots are popularly used as remedial against stings of rays, muscle pain, wounds, and stomach problems. When burned with other herbs, this species produces smoke (mafusho), which a patient inhales as vapors to cure fever by lowering body temperature. Another species, *Cymodocea* spp., is utilized to combat malaria and cough (De La Torre-Castro and Rönnbäck 2004). *T. ciliatum* is known for its effectiveness in treating various ailments (Abdelhameed et al. 2018). Furthermore, combinations of seagrasses, including *Thalassia* and *Cymodocea*, are recognized for their use in treating fever and skin diseases. In a previous study, Hamisi et al. (2023) reported that extracts from seven common seagrass species—*C. serrulata*, *H. uninervis*, *E. acoroides*, *C. rotundata*, *S. isoetifolium*, *T. hemprichii*, and *T. ciliatum*—exhibited potential antibacterial activities. In this study, we further analyzed the phytocompound composition of these seagrasses using Gas Chromatography-Mass Spectrometry to complement the existing knowledge and provide scientific evidence for their potential in pharmaceutical development.

MATERIALS AND METHODS

Seagrasses collection

Seven seagrass species, namely *C. serrulata*, *H. uninervis*, *E. acoroides*, *C. rotundata*, *S. isoetifolium*, *T. hemprichii*, and *T. ciliatum*, were collected during low tides from the coastal areas of Mjimwema (06°50'S, 39°21'E), 4 km south of the Dar es Salaam harbor, and from Bagamoyo (6°27'32"S, 38°56'E) between the Bagamoyo fish landing site and Kaole ruins (Figure 1). Seagrasses were identified in the field and the laboratory using standard identification guidebooks (Oliveira et al. 2005; Richmond 2011). Sampling was done by uprooting seagrasses using a shovel. The seagrass leaves (phyllosphere) and the below-ground parts (roots/rhizome) were separated using scissors and transported to the University of Dar es Salaam, Department of Molecular Biology and Biotechnology laboratories for processing and analysis.

Preparation of seagrasses for analysis

In the laboratory, the collected seagrass samples were washed twice to three times with fresh water to remove debris. Both leaves and roots were air dried under shade for 10 to 14 days to a constant weight (Figure 2) and then mechanically pulverized to obtain a fine powder using a Thomas Wiley Laboratory mill model 4 (Philadelphia, USA).

Preparation of extracts

Approximately 100 g of the powdered sample of each species was soaked in 500 mL of *n*-hexane at room temperature in a shaker (Edmund Buhler 7400) for 48 hours. The filtrates were then subjected to a rotary evaporator (BUCHI rotary vapor model R-210) to evaporate the solvent and get the crude extract (Kannan et al. 2010b). Table 1 displays the quantity of extracts obtained from each seagrass species following solvent extraction. The extracts were stored at 4°C before analysis using Gas Chromatography-Mass Spectrometry (GC-MS).

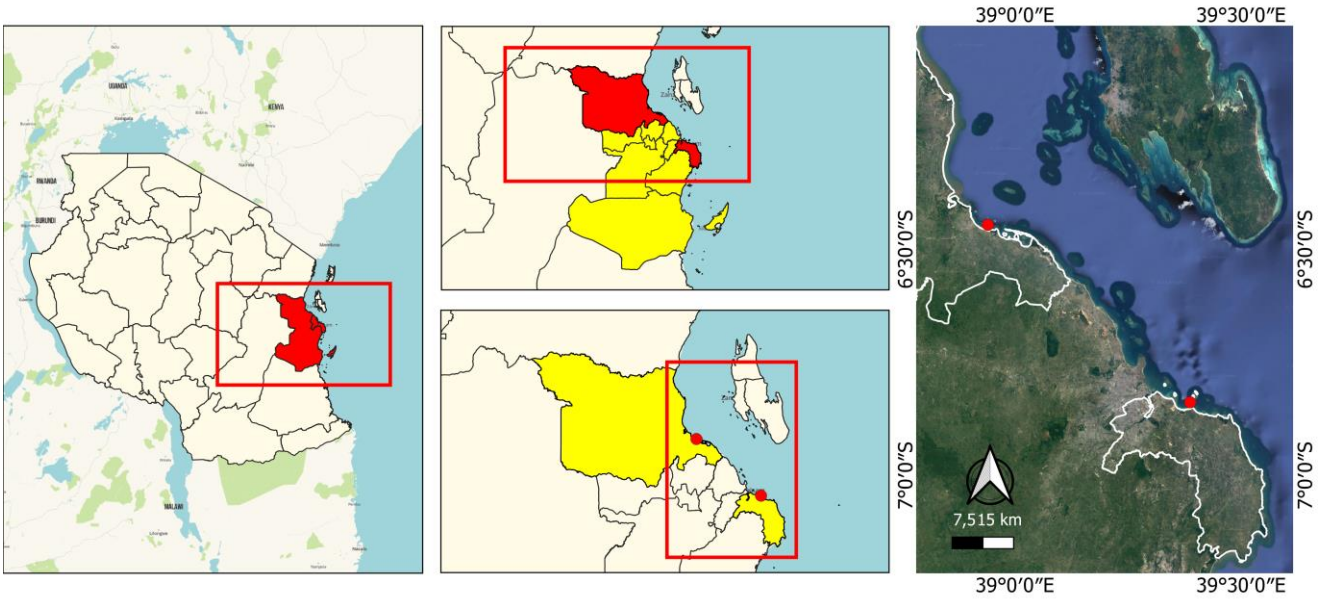


Figure 1. A map showing sampling stations at Mjimwema and Bagamoyo, Tanzania

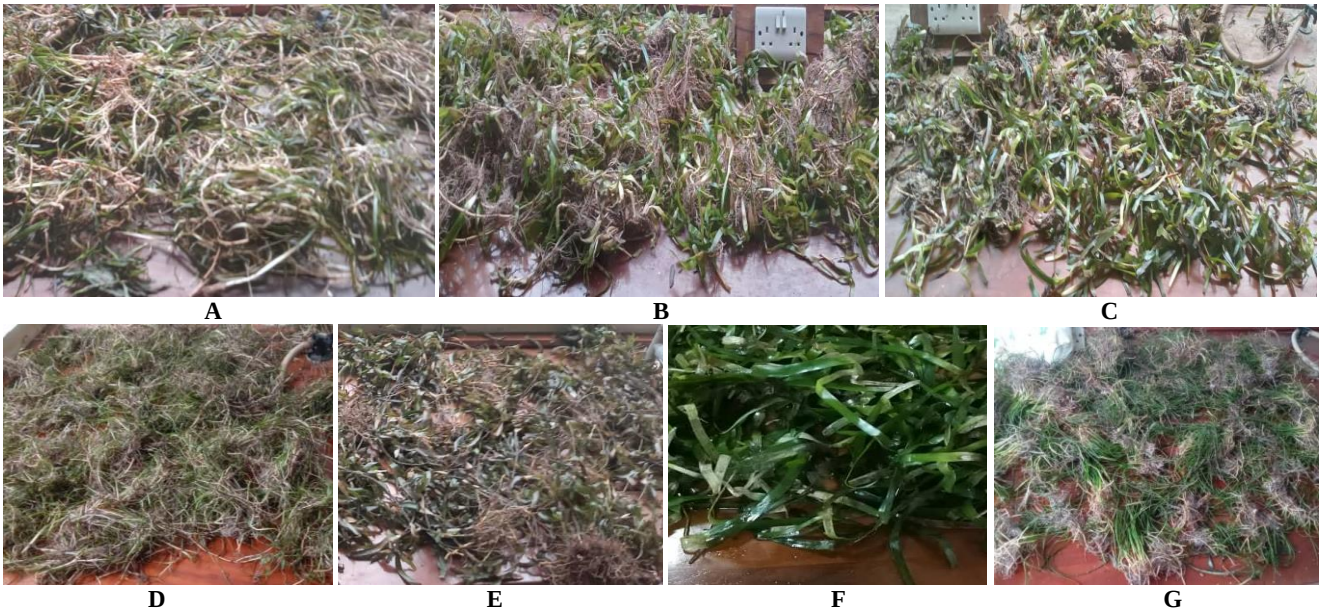


Figure 2. Photographs showing the seven seagrass species on laboratory benches to attain a constant weight: A. *C. rotundata*, B. *C. serrulata*, C. *E. acoroides*, D. *H. uninervis*, E. *T. ciliatum*, F. *T. hemprichii* and, G. *S. isoetifolium*

Table 1. Amount of extracts yield for each seagrass species

Seagrass species	Leaves /roots	Amount of powder (g)	Amount of solvent (ml)	Extracts yield (g)
C. rotundata	Leaves	100	500	0.513
	Roots	100	500	0.274
C. serrulata	Leaves	100	500	0.541
	Roots	100	500	0.523
H. uninervis	Leaves	100	500	0.561
	Roots	NA	NA	NA
T. hemprichii	Leaves	100	500	0.830
	Roots	100	500	0.235
T. ciliatum	Leaves	100	500	0.377
	Roots	100	500	0.226
S. isoetifolium	Leaves	100	500	0.605
	Roots	100	500	0.360
E. acoroides	Leaves	NA	NA	NA
	Roots	100	500	0.1

Note: NA: Not Analysed

Gas Chromatography-Mass Spectrometry analysis

Analysis of the extracts was done by GC-MS, whereby 1 µL of the crude extract in 1 mL of dichloromethane was injected into the instrument. The peaks were recorded in a GCMS-QP 2010 Ultra (Shimadzu instrument) operating in electron ionization (EI) mode (MS) at 70 eV and a flame ionization detector (FID) for GC. A Restek-5MS column (30 m x 0.25 mm x 0.25 µm) was used. The oven temperature program was 90°C to 280°C and was held at 90°C for 2 minutes. The temperature was increased to 280°C for 8 minutes (hold time) at 6°C per minute. The injection temperature was 250°C with split injection mode. Helium was used as carrier gas at a flow rate of 1.21 mL min^{-1}. The ion source temperature and interface temperature in MS were 230°C and 300°C, respectively. The identification of compounds in the sample was performed via the scan method, which involved the use of the Mass Spectral Library & Search Software (NIST 11). The quantification of the compounds in the samples was then performed based on percentage composition using the peak integration method, whereby the ion allowance was 20% (Elkhateeb et al. 2019).

RESULTS AND DISCUSSION

GC-MS revealed biologically active phytocompounds

GC-MS is often referred to as the "gold standard" for analytical substance identification because it is highly effective for specific test analysis (Perez et al. 2016). GC-MS can be used to identify various substances within a test sample, including hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds, and can be used to identify trace amounts of materials (Saravanan et al. 2014). This study revealed the presence of 24 different biologically active phytocompounds from the hexane extracts of the seven seagrass species, as shown in Table 2 and Supplementary Material Figures S.1-12. Furthermore, the most common phytocompounds observed in all seagrass species, except for *S. isoetifolium*, were stigmasta-5,22-dien-3-ol acetate and 3-β, β-sitosterol acetate. The abundance of stigmasta-5,22-dien-3*β*-ol acetate ranged from 5.01 to 62.4% and 1.08 to 22.3% for the seagrass leaves and root extracts, respectively. The 3*β* sitosterol acetate abundance ranged from 2.69 to 11.6% and 0.88 to 16.7% for the leaf and root extracts, respectively. These results are similar to other findings that have reported the same compounds in various seagrass species, including *Cymodocea serrulata* (Pushpabharathi et al. 2018). Stigmasta-5,22-dien-3*β*-ol acetate and 3*β*-sitosterol acetate

have been reported to possess various bioactive properties, as summarized in Table 3. The presence of these compounds may, therefore, explain and support the local use of the seagrass species in the WIO region for various treatments. Analysis of the results from different seagrass species revealed that they contain different phytocompounds with various compositions, as presented in the following sections.

Phytocompounds from *Cymodocea serrulata* extracts

Eight (8) compounds were identified in both the leaves and roots of seagrass *C. serrulata* extracts. The prevailing compounds in the leaves were stigmasta-5,22-dien-3*β*-ol, acetate (62.4%), 6,10,14-trimethyl-2-pentadecanone (13.0%), and 3*β*-sitosterol acetate (11.6%), whereas cyclic octaatomic sulfur (65.3%), heptadecane (12.0%) and hexadecane (10.2%) were found in the roots (Table 2). Among these compounds, stigmasta-5,22-dien-3*β*-ol, acetate, 2-pentadecanone, and 6,10,14-trimethyl-, have been reported on the same seagrass from India by Pushpabharathi et al. (2018). However, Pushpabharathi et al. (2018) reported five compounds using ethanol extracts while Das et al. (2023) reported the compounds hexadecane and heptadecane among other compounds from the same seagrass that revealed a total of 104 phytocompounds from chloroform, ethanol, and distilled water from *C. serrulata* samples from Palk Bay, India. Moreover, other researchers have detected several compounds from this seagrass species, but none were similar to the compounds extracted from this study. For example, Vijayalingam and Rajesh (2019) revealed nine compounds using ethanol extracts, and Jeevith et al. (2019) detected 26 compounds from the same seagrass using methanol extracts. Thus, to our knowledge, in this study, six compounds from seagrass *C. serrulata* extracts have been identified for the first time.

Phytocompounds from *Cymodocea rotundata* extracts

The extracts of the seagrass *C. rotundata* were found to contain 10 phytocompounds from both the leaves and roots. The main chemical components in the leaves were stigmasta-5,22-dien-3*β*-ol acetate (41.9%), pentadecanal (28.7%), and 6,10,14-trimethyl-2-pentadecanone (16.0%), whereas in the roots, the main chemical compounds were cyclic octaatomic sulfur (56.0%), heptadecane (15.6%), and hexadecane (13.4%). The steroid cholest-5-en-3-ol (3*β*)-, tetradecanoate was uniquely obtained from this seagrass. None of the revealed phytocompounds match the compounds reported by Perez et al. (2018), who used dichloromethane extract on the same seagrass. Hence, the current study has identified ten compounds previously unreported from *C. rotundata*.

Phytocompounds from *Halodule uninervis* extracts

In the seagrass, *H. uninervis* extracts, 13 phytocompounds were found in leaves, as shown in Table 1. The significant phytocompounds detected in *H. uninervis* were tetracosane (33.4%), 1-nonadecene (13.1%), and *β*4-sitosterol-3-one (15.5%). Interestingly, among the studied species, steroid compounds *β*4-sitosterol-3-one and cholestadien-3-one were detected only in *H. uninervis* (Table 2). However, the revealed phytocompounds have never been detected in this seagrass species. Using ethyl acetate, Parthasarathi et al. (2021) reported 23 phytocompounds from the same seagrass *H. uninervis* extracts, but none of the compounds identified matched those releaved in this study.

Phytocompounds from *Enhalus acoroides* extracts

The GC-MS analysis of *E. acoroides* revealed only four compounds. The major phytocompounds identified from this seagrass were cyclic octaatomic sulfur (97.4%), followed by stigmasta-5,22-dien-3*β*-ol acetate (1.08%). Out of the four compounds, cyclic octaatomic sulfur has previously been reported from the same species by Selvam et al. (2022). The study by Amudha et al. (2018) revealed 29 phytocompounds from ethyl acetate crude extracts of this seagrass species from Devipattinam, Ramanathapuram, India. Another study by Vijayalingam and Rajesh (2019) reported 10 phytocompounds from ethanol extracts from the same seagrass species found in India. In both cases, none of the reported compounds were the same as these hereby reported.

Phytocompounds from *Syringodium isoetifolium* extracts

The seagrass *S. isoetifolium* extracts were found to contain six phytocompounds in both the leaves and roots. The major phytocompounds in the leaves were 2,4-di-tert-butylphenol (30.9%), 1-pentadecene (17.1%), and 1-tridecene (16.0%), while in the roots the notable compounds were 2,4-di-tert-butylphenol (30.9%), 1-pentadecene (18.2%), and 1-tridecene (16.6%). All six revealed phytocompounds have not been reported from this species elsewhere. The study by Vijayalingam and Rajesh (2019) revealed eight compounds from ethanol of the same species collected from Seeniyappa Dharka, India, while that conducted by Jeevitha et al. (2019) reported 25 compounds from methanol of the species obtained from the Gulf of Mannar, Tamil Nad. However, none of these previous studies reported the same compounds obtained in this present study.

Phytocompounds from *Thalassia hemprichii* extracts

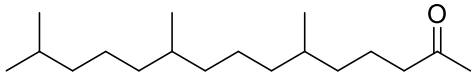
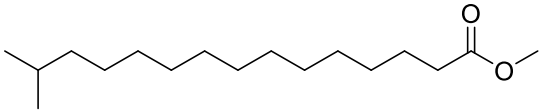
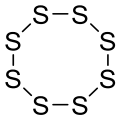
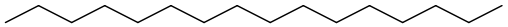
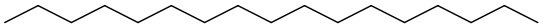
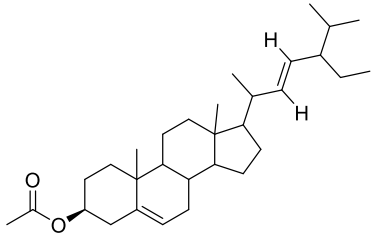
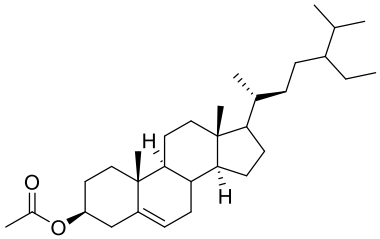
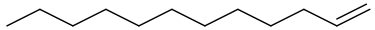
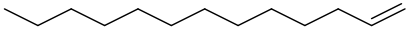
Nine phytocompounds were detected in the leaves and roots of the seagrass *T. hemprichii* hexane extracts. The significant phytocompounds from the leaves were cyclic octaatomic sulfur (54.0%), stigmasta-5,22-dien-3β-ol acetate (10.6%), and *n*-hexadecanoic acid (8.7%). In comparison, in the roots, the significant phytocompounds were cyclic octa atomic sulfur (63.5%), 1-pentadecene (6.86%), and 3*β*-sitosterol acetate (6.19%). Furthermore, the fatty acid (*n*-hexadecanoic acid) was found only in *T. hemprichii*. None of the phytocompounds hereby revealed have been reported from the same seagrass species growing elsewhere. For example, Hassan et al. (2022) analyzed *T. hemprichii* collected from the Red Sea, Egypt and reported the existence of nine phytocompounds from hexane extract, but none of the compounds are similar to those found in this study.

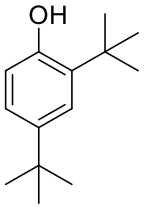
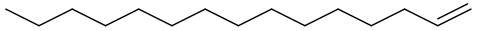
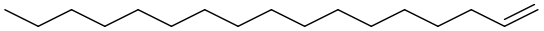
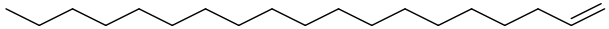
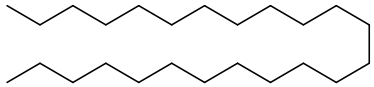
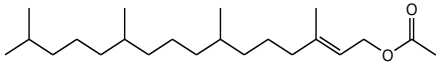
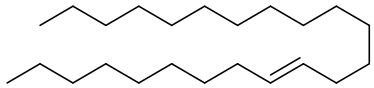
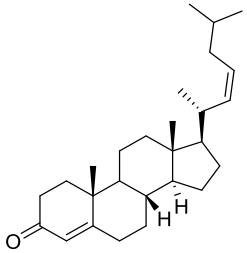
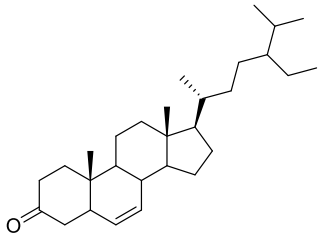
Table 2. Phytocompounds identified in hexane extracts of seven seagrass species and their relative abundance

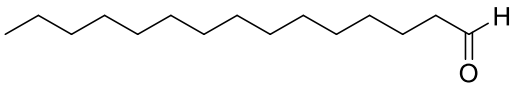
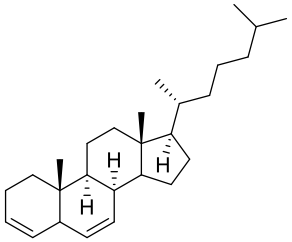
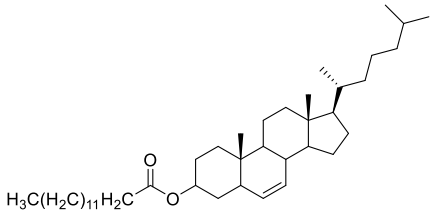
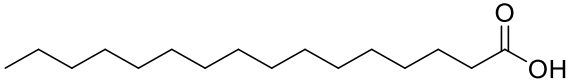
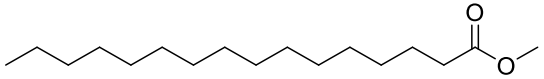
SN	RT	Name of the compound	Formula	MW	Peak Area %													
					CS		HU		EA		CR		SI		TH		TC	
					L	R	L	R	L	R	L	R	L	R	L	R	L	R
1	16.76	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268.0	13.0	0.85	-	-	NA	-	16.0	0.46	-	-	-	-	2.67	-
2	18.12	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270.0	6.91	3.16	-	-	-	-	4.76	2.14	-	-	-	-	-	0.33
3	19.52	Cyclic octaatomic sulfur	S_8	256.5	-	65.3	-	-	NA	97.4	-	56.0	-	-	53.97	63.50	-	55.4
4	20.82	Hexadecane	$C_{16}H_{36}$	226.4	-	10.2	-	-	NA	-	-	13.4	-	-	-	-	-	0.74
5	23.69	Heptadecane	$C_{17}H_{36}$	240.5	-	12.0	-	-	NA	0.62	-	15.6	-	-	-	-	-	0.16
6	35.03	Stigmasta-5,22-dien-3β-ol, acetate	$C_{31}H_{50}O_2$	454.0	62.4	6.92	5.01	NA	NA	1.08	41.9	5.75	-	-	10.95	6.19	-	22.3
7	35.79	3β-Sitosterol acetate	$C_{31}H_{52}O_2$	456.7	11.6	1.57	3.18	NA	NA	0.88	7.17	5.81	-	-	2.69	3.88	-	16.7
8	4.58	1-Dodecene	$C_{12}H_{24}$	168.3	-	-	0.69	NA		-	-	-	10.8	10.5	4.44	4.08	2.37	-
9	8.37	1-Tridecene	$C_{13}H_{26}$	162.2	-	-	1.17	NA	-	-	-	-	16.0	16.6	5.43	6.10	3.22	-
10	10.78	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.3	-	-	1.32	NA	-	-	-	-	35.2	30.9	-	-		
11	12.27	1-Pentadecene	$C_{15}H_{30}$	210.4	-	-	1.43	NA	-	-	-	-	17.1	18.2	6.58	6.86	3.57	-
12	15.89	1-Heptadecene	$C_{17}H_{34}$	238.5	-	-	2.12	NA	-	-	-	-	12.2	14.7	5.34	5.66	3.26	-
13	19.18	1-Nonadecene	$C_{19}H_{38}$	266.5	-	-	13.1	-	-	-	-	-	-	-	-	-	2.87	-
14	20.81	Tetracosane	$C_{24}H_5O$	338.7	-	-	33.4	-	-	-	-	-	-	-	-	-	14.9	-
15	21.01	Phytol, acetate	$C_{22}H_{42}O_2$	256.5	-	-	12.0	-	-	-	-	-	-	-	-	-	17.6	-
16	22.19	(*Z*)-9-Tricosene	$C_{23}H_{46}$	322.6	-	-	3.60	-	-	-	-	-	8.72	9.09	-	-	-	-
17	36.88	4,22-Cholestadien-3-one	$C_{27}H_{42}O$	382.6	-	-	6.79	-	-	-	-	-	-	-	-	-	-	-
18	37.85	β 4-Sitosterol-3-one	$C_{29}H_{48}O$	412.7	-	-	15.5	-	-	-	-	-	-	-	-	-	-	-
19	21.01	Pentadecanal-	$C_{15}H_{30}O$	226	-	-	-		-	-	28.7	-	-	-	-	-	-	-
20	33.41	Cholesta-3,5-diene	$C_{27}H_{44}$	368	6.10	-	-		-	-	1.46	-	-	-	1.86	1.00	0.93	-
21	34.62	Cholest-5-en-3-ol (3β)-, tetradecanoate	$C_{41}H_{72}O_2$	386.6	-	-	-	-	-	-	-	0.81	-	-	-	-	-	4.44
22	18.71	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	-	-	-	-	-	-	-	-	-	-	8.71	2.72	-	-
23	17.68	Heneicosane	$C_{21}H_{44}$	296.5	-	-	-	-	-	-	-	-	-	-	-	-	42.1	-
24	18.12	Hexadecanoicacid, methyl ester	$C_{17}H_{34}O_2$	270	-	-	-	-	-	-	-	-	-	-	-	-	6.52	-

Note: -: Absent, NA: Not Determine, RT: Retention Time, MW: Molecular Weight, L: Leaves, R: Roots, CS: *C. serrulata*, HU: *H. uninervis*, EA:*E. acoroides*, CR: *C. rotundata*, SI*: S. isoetifolium*, TH: *T. hemprichii* and TC: *T. ciliatum*

Table 3. Biological activities of reported compounds in the hexane extracts of seagrasses

SN	Compound name	Structure	Nature of compound	Biological activity
1	2-Pentadecanone, 6,10,14-trimethyl-		Essential oil	Antihypertensive activity and vasodilatation affect cerebral and basilar arteries (Pushpabharathi et al. 2018)
2	Pentadecanoic acid, 14-methyl-, methyl ester		Fatty acids	Many odd-length amino acids are derived from pentadecanoic acid. Act as a biological marker for dietary milk intake (Pushpabharathi et al. 2018)
3	Cyclic octa atomic sulfur		Sulfur	Pesticide (Steudel and Chivers 2019)
4	Hexadecane		Alkane hydrocarbon	Antibacterial (Kumaresan et al. 2015)
5	Heptadecane		Straight chain alkane	Antifungal (Abubackerand and Devi 2015)
6	Stigmasta-5,22-dien-3β -ol, acetate		Steroids	Antiosteoarthritic, Antihypercholesterolemi, cytotoxicity, antitumour, hypoglycaemic, antimutagenic, antioxidant, Anti-inflammatory, and CNS effects (Kaur et al. 2011)
7	3β-Sitosterol acetate		Steroids	Anti-inflammatory, antineoplastic, antipyretic, and immunomodulating activity (Amudha et al. 2018)
8	1-Dodecene		Alkene	Antibacterial (Togashi 2007)
9	1-Tridecene		Alkene	Antibacterial activity (Kumar et al. 2011)

10	2,4-Di-tert-butylphenol		Phenol	Antifungal (Sang and Kim 2012), Antimalarial (Kusch et al. 2011)
11	1-Pentadecene		Alkene	Antimicrobial (Dubal et al. 2015)
12	1-Heptadecene		Alkene	Artificial ripening of fruits, metabolism of aromatic compounds in animals antimicrobial (Lakshmi and Bindu 2017)
13	1-Nonadecene		Alkene	Antituberculosis, anticancer, antioxidant, antimicrobial (Amudha et al. 2018)
14	Tetracosane		Straight chain alkane	Cytotoxicity against cancerous cells, Antidiarrheal, Antibacterial, Cardiotonic, laxative, Anthelmintic and removes fatigue, Anti-inflammatory, used in peptic ulcer treatment, Anticorrosive, Antioxidant, and Antitrichomonas (Prabhanna and Jayaraj 2018)
15	Phytol acetate		Diterpene	Antimicrobial activity (Mundt et al. 2003)
16	9-Tricosene		Alkene	Housefly pheromone (Gołębiowskia et al. 2015)
17	4,22-Cholestadien-3-one		Cholesterol	Anticancer (Patil and Singh 2022)
18	β4-Sitosterol-3-one		Steroids	Anticancer (Singariya et al. 2014)

19	Pentadecanal		Long-chain fatty aldehyde	Antimicrobial, a volatile oil component and a plant metabolite (Ogunwande et al. 2013)
20	Cholesta-3,5-diene		Cholesterol	Wound healing property and skin protector activities (Sayik et al. 2017)
21	3β -Cholest-5-en-3-ol, tetradecanoate		Steroids	Antibacterial, antioxidant (Thanigaivel et al. 2015)
22	*n*-Hexadecanoic acid		Palmitic acid	Antioxidant, hypocholesterolemic, nematicide, ant-androgenic hemolytic, and Alpha reductase inhibitor (Vijayalingam and Rajesh 2019)
23	Heneicosane		Straight chain alkane	Antioxidant and antimicrobial (Nimbeshaho et al. 2020)
24	Hexadecanoicacid, methyl ester		Fatty acids	Antibacterial and antifungal activity (Wagh et al. 2006)

Phytocompounds from *Thalassodendron ciliatum* extracts

The seagrass *T. ciliatum* had more phytocompounds compared to other studied seagrass species. Eighteen phytocompounds were found in this seagrass species, of which eleven and seven were found in the leaf and root extracts, respectively. The predominant phyto-compounds from the leaves were heneicosane (42.1%), phytol, acetate (17.6%), and tetracosane (14.9%), while those from the roots were cyclic octaatomic sulfur (55.4%), 3*β*-sitosterol acetate (22.3%), and 1-dodecene (16.7%). Furthermore, hydrocarbon (heneicosane) and fatty acid (hexadecanoic acid, methyl ester) were found only in the leaves of this seagrass. Of the 18 revealed phytocompounds, only one compound, fatty acid methyl ester (14-methyl-pentadecanoic acid methyl ester), was reported from another study conducted in the Red Sea, Egypt, by Goda et al. (2020), identifies 21 compounds from similar seagrass extracted using the same solvent (hexane).

Discussion

As observed in this study, the differences in the composition of the phytocompounds could be attributed to differences in geographical location, the type of seagrass species, and the solvent used. For instance, the variations in phytocompounds in similar seagrass species extracted using similar solvents are most likely due to differences in geographical location (Khanzadi and Tajur 2015). Despite the differences resulting from similar seagrass species, the different solvents used could be attributed to differences in polarity. The solvent used in this study was hexane, which is nonpolar as opposed to the polar solvents used by most of the researchers above. Additionally, the mineral composition, soil type, temperature, light, and water content are frequently reported factors affecting plant total phytochemical contents (Rao and Rao 2007; Hansen et al. 2010).

A comparison of the seven seagrasses from our study revealed that the seagrass *T. ciliatum* had more phytocompounds compared to other studied seagrass species, whereas hydrocarbon (heneicosane) and fatty acid (hexadecanoic acid, methyl ester) were found only in the leaves of this seagrass, and they are known for their antimicrobial and antioxidant properties (Wagh et al. 2006; Nimbeshaho et al. 2020). The seagrass *T. ciliatum* is traditionally popular for the treatment of smallpox and fever (De la Torre-Castro and Rönnbäck 2004); this function corresponds to the functions of 3*β*-sitosterol acetate, 1-dodecene, 1-tridecene, and hexadecane. Additionally, the steroid compounds *β*4-Sitosterol-3-one and 4,22-cholestadien-3-one, which are known for their antineoplastic activities (Singariya et al. 2014; Patil and Singh 2022) were detected only in *H. uninervis*. Furthermore, the steroid cholest-5-en-3-ol (3*β*)-, tetradecanoate was only obtained from *C. rotundata* and is known to have bioactivities (Thanigaivel et al. 2015). Traditionally, *Cymodocea* spp. have been reported to be used as remedies for skin diseases, fever, and cough and are believed to help during pregnancy as tranquilizers for babies (De la Torre-Castro and Rönnbäck 2004). These findings correlate well with the bioactivities of the identified compounds, such as 3*β*-sitosterol acetate, cholesta-3,5-diene, heptadecane, and pentadecanal, which are known for their antipyretic and wound healing properties, skin protector activities, and antifungal and antimicrobial activities (Table 2). The fatty acid (n-hexadecanoic acid), a compound reported to exhibit various bioactive properties (Vijayalingam and Rajesh 2019), was found exclusively in *T. hemprichii*. Additionally, compounds such as heptadecane, stigmasta-5,22-dien-3*β*-ol acetate, and 3*β*sitosterol acetate are recognized for their antimicrobial properties and their ability to reduce fever. Kaur et al. (2011), Abubackerand and Devi (2015), and Amudha et al. (2018) are found in the roots of *E. acoroides*. The anticipated function of the compounds mentioned above correlates well with the traditional use of seagrass. Thus, *E. acoroides* have been widely used against stings of special kinds of rays and scorpions (Kannan et al. 2010b) as well as against fever, muscle pains, wounds, and stomachs (De la Torre-Castro and Rönnbäck 2004).

Generally, seagrasses are rich in antimicrobial, antioxidant, and anti-inflammatory compounds due to the phytocompounds identified. They have therefore been reported to display antibacterial (Hamisi et al. 2023), antifungal, antimalarial (Kim et al. 2021), antioxidantactivities, increased glutathione sulfotransferase (GST) enzyme activity, lipid peroxidation, ferric reducing (Kim et al. 2021), and anti-Inflammatorymuscle aches, wounds, and abdominal pain (Kim et al. 2021).

The present GC-MS analysis revealed multivarious bioactive compounds with diverse chemical structures among which stigmasta-5,22-dien-3*β*-ol acetate and 3*β*-sitosterol acetate were the most common. The structures of the identified compounds and their corresponding known biological activities are presented in Table 3. The compounds 3*β*-Sitosterol acetate and stigmasta-5,22-dien-3*β*-ol acetate are reported to be steroids derived from lanosterol, a tetracyclic triterpenoid that can be used to produce oxy-generated derivatives of cholesterol and lanosterol. This finding demonstrated that oxysterols may be natural regulators of cholesterol biosynthesis in intact cells (Wang et al. 2008). Phytol acetate, a diterpene, has several biological activities such as anti-inflammatory, antimicrobial and antispasmodic activities. Furthermore, diterpenes have been shown to exhibit cardiovascular effects (Tirapelli et al. 2008). In this study, straight-chain alkanes such as heptadecane, were reported from *C. serrulata*, *E. acoroides*, and *C. rotundata* and are known to have antimicrobial properties (Vijayalingam and Rajesh 2019). This alkane was previously reported in *C. serrulata* by Das et al. (2023) from Palk Bay, India.

Generally, the findings of this study revealed the presence of useful chemical compounds such as essential oils, fatty acids, steroids, cholesterol, phenol, diterpenes, straight chain alkanes, and alkenes from seven seagrass species found along the Tanzania coast of the Western Indian Ocean, some of which are known for different bioactivities that reflect the traditional use of these seagrasses. A total of 24 known phytocompounds were identified across the examined species, with notable

common compounds including stigmasta-5,22-dien-3-ol acetate and 3β-sitosterol acetate. Additionally, certain unique compounds, such as 3β-cholest-5-en-3-ol tetradecanoate and phytol acetate were found in specific species. The *T. ciliatum*, in particular, exhibited a greater variety of compounds compared to the other seagrass species studied. This research is the first to report fourteen compounds in these seagrass species, underscoring their potential as sources of pharmacologically significant substances with antimicrobial, antioxidant, and anti-inflammatory properties. The findings contribute to the frontier of knowledge on phytochemicals present in seagrass species growing in the Western Indian Ocean, a scarcity explored ecosystem. We recommend further assessment of the efficacy and safety of these phytocompounds through in vitro and in vivo studies to understand their mechanisms of action and potential therapeutic applications. Additionally, to fully elucidate the spectrum of bioactive compounds present, we suggest exploring other extraction methods and analytical techniques to identify additional compounds.

ACKNOWLEDGEMENTS

The authors appreciate the University of Dar es Salaam (UDSM), Tanzania for providing financial support to carry out this research for the benefit and well-being of the scientific community through project number CoNAS MBB 20036. The authors are also pleased to extend their genuine thanks to Dr. Fortunatus Sungwa, a chief laboratory scientist at the Chemistry Department, UDSM, for help in performing the GC-MS analysis and Prof. Stephen Nyandoro for his valuable contributions to scientific writing methods and language editorials.

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SUPPLEMENTARY MATERIAL:
REPRESENTATIVE CHROMATOGRAM OF HEXANE EXTRACTS OF SEVEN SEAGRASSES

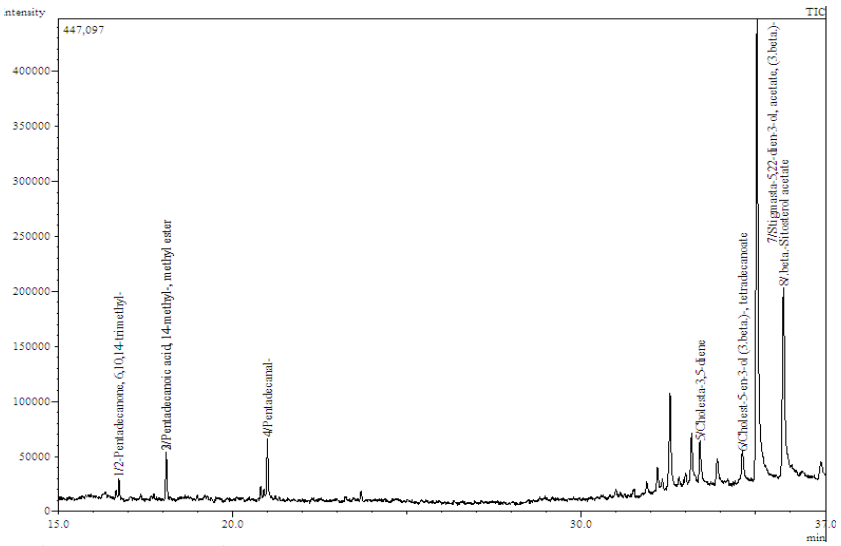

Figure S1. Chromatogram of hexane extracts of *Cymodocea rotundata* leaves

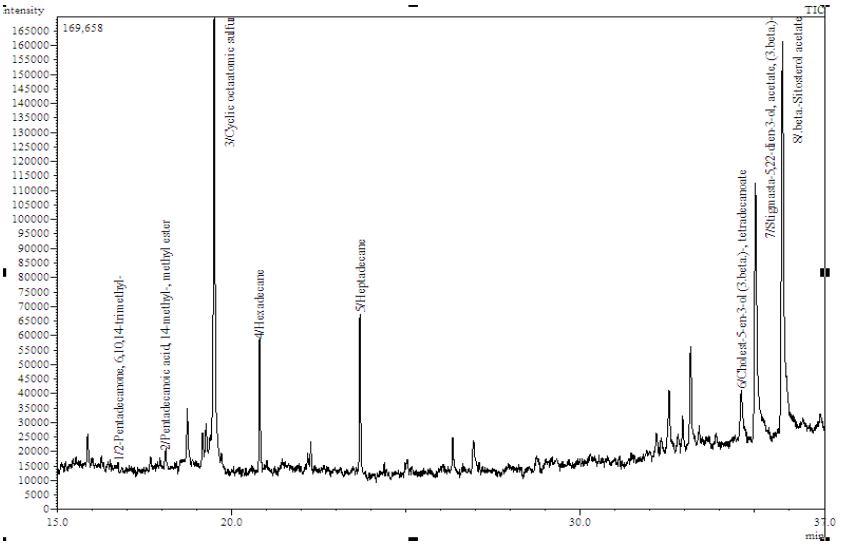

Figure S2. Chromatogram of hexane extracts of *Cymodocea rotundata* roots

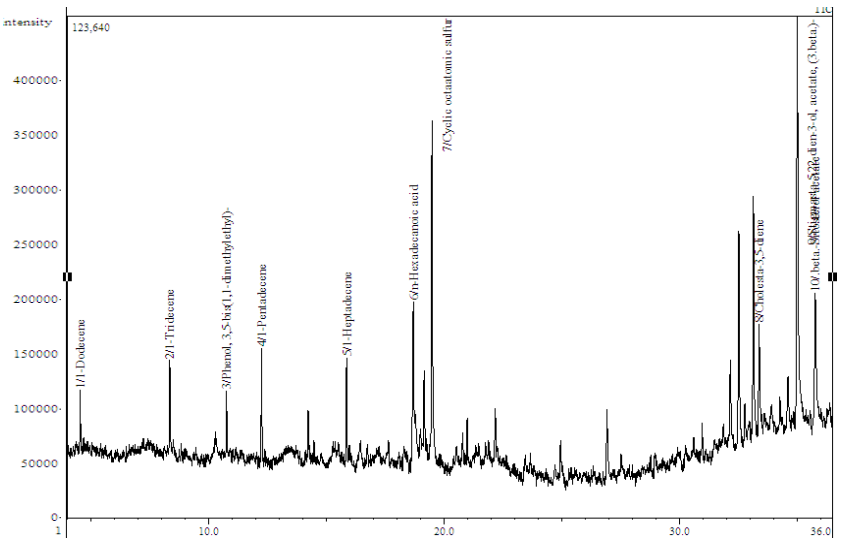

Figure S3. Chromatogram of hexane extracts of *Thalassia hemprichii* leaves

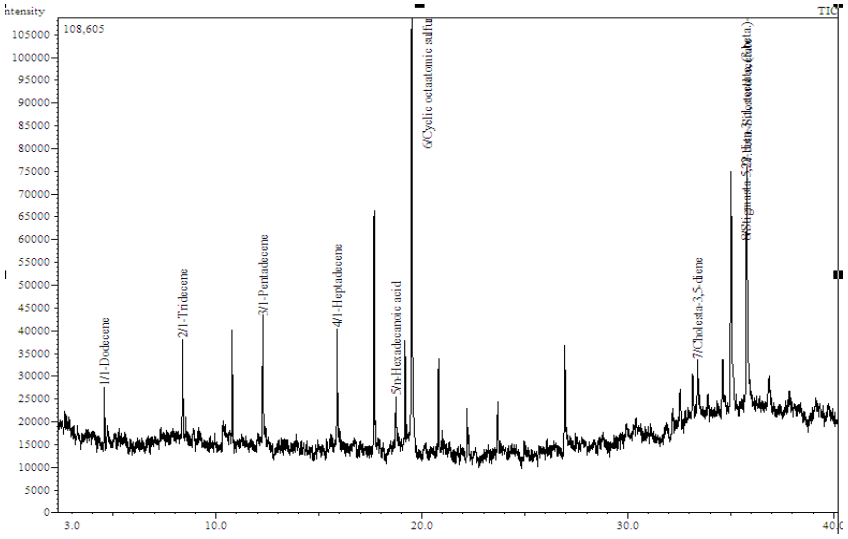

Figure S4. Chromatogram of hexane extracts of *Thalassia hemprichii* roots

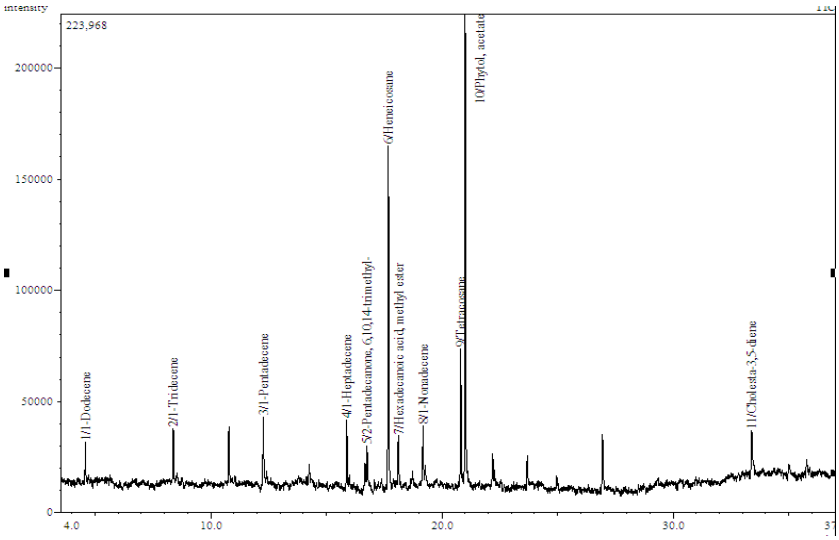

Figure S5. Chromatogram of hexane extracts of *Thalassodendron ciliatum* leaves

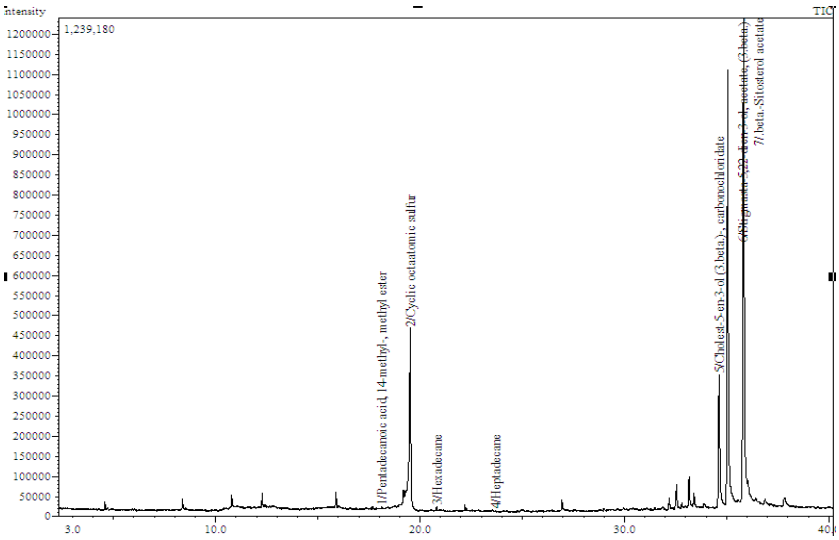

Figure S6. Chromatogram of hexane extracts of *Thalassodendron ciliatum* roots

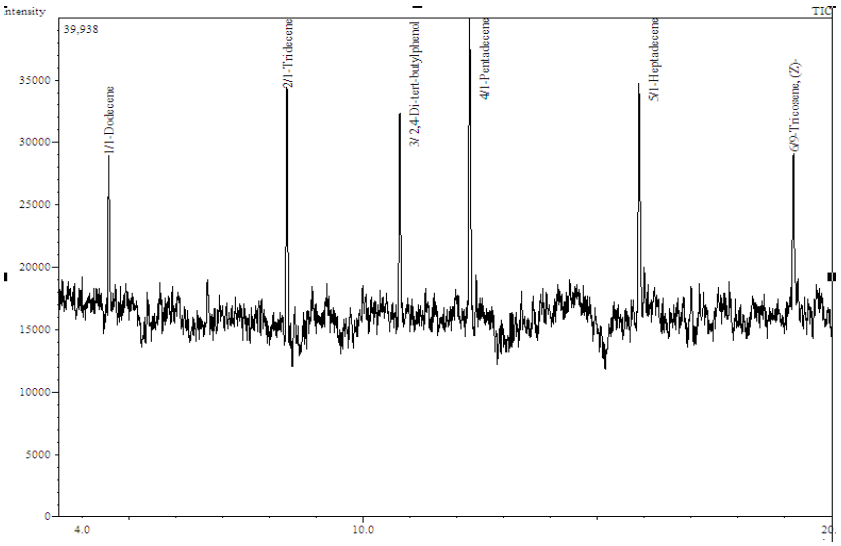

Figure S7. Chromatogram of hexane extracts of *Syringodium isoetifolium* leaves

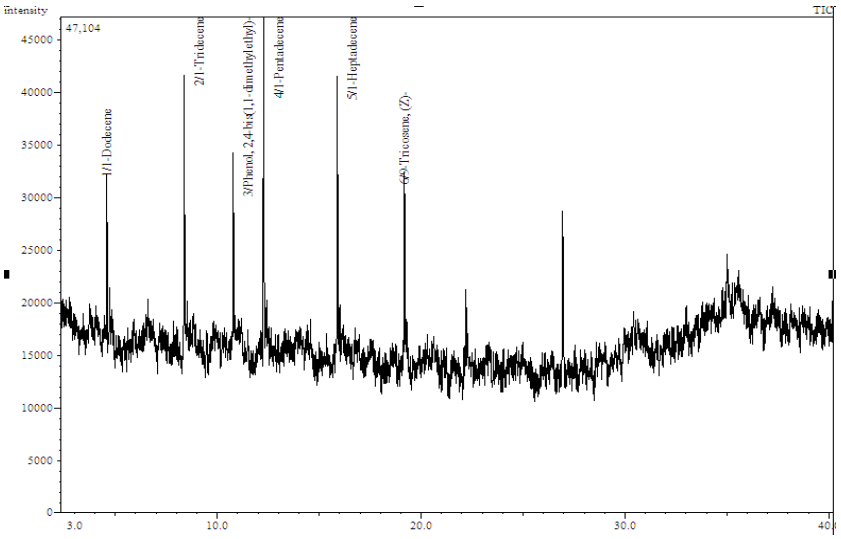

Figure S8. Chromatogram of hexane extracts of *Syringodium isoetifolium* roots

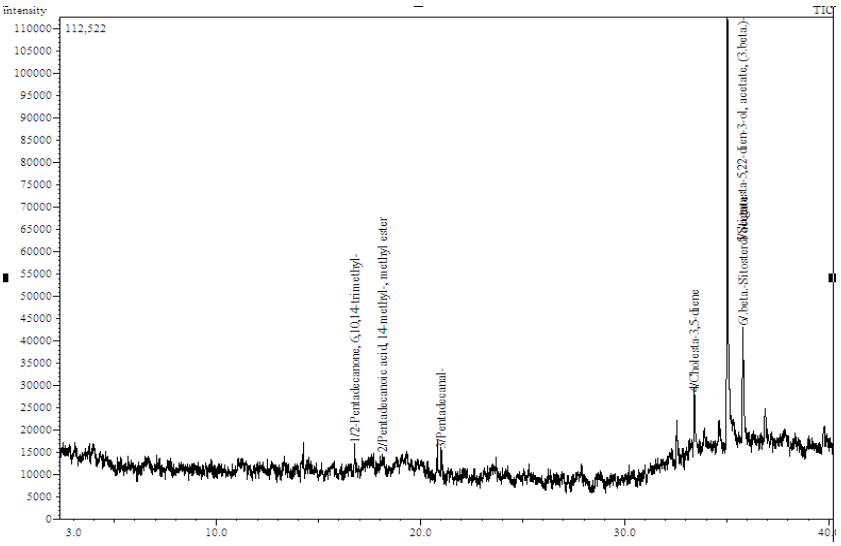

Figure S9. Chromatogram of hexane extracts of *Cymodocea serrulata* leaves

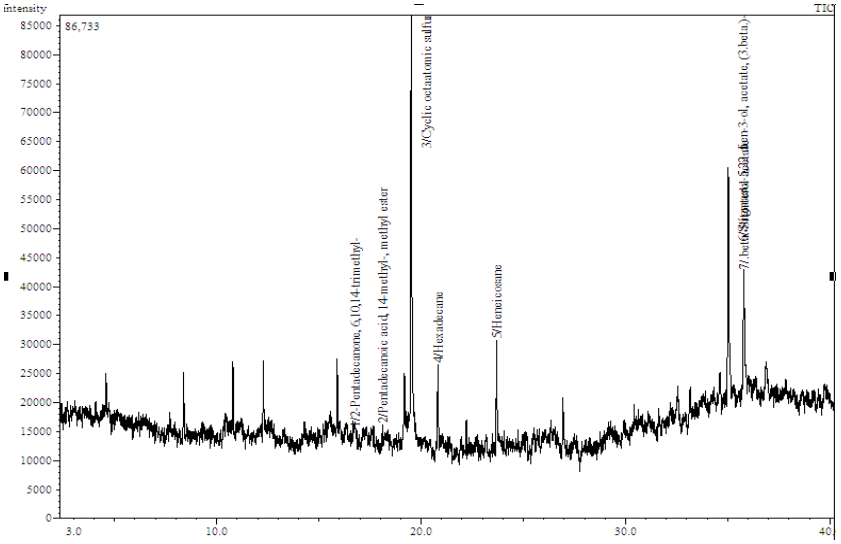

Figure S10. Chromatogram of hexane extracts of *Cymodocea serrulata* roots

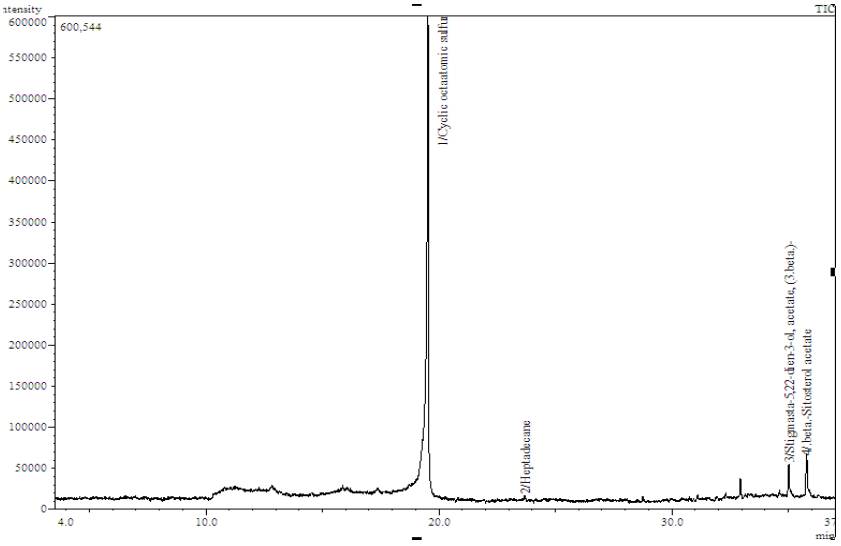

Figure S11. Chromatogram of hexane extracts of *Enhalus acoroides* roots

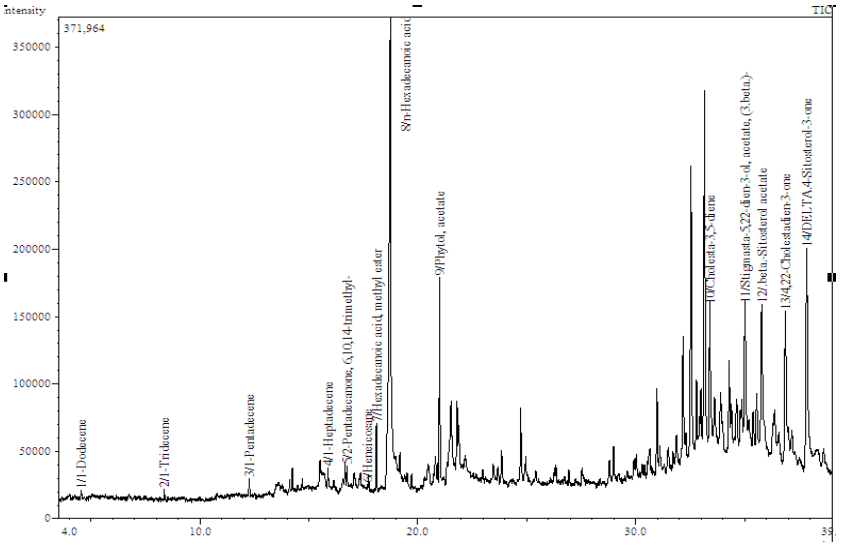

Figure S12. Chromatogram of hexane extracts of *Halodule uninervis* roots