marine drugs

MDPI

Supplemental material

LC-ESI-QTOF-MS/MS Characterization of seaweed phenolics and their antioxidant potential

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Abstract: Seaweed is an important food widely consumed in Asian countries. Seaweed has a diverse array of bioactive compounds, including dietary fiber, carbohydrate, protein, fatty acid, minerals and polyphenols, which contribute to the health benefits and commercial value of seaweed. Nevertheless, detailed information on polyphenol content in seaweeds is still limited. Therefore, the present work aimed to investigate the phenolic compounds present in eight seaweeds [Chlorophyta (green), *Ulva* sp., *Caulerpa* sp. and *Codium* sp.; Rhodophyta (red), *Dasya* sp., *Grateloupia* sp. and *Centroceras* sp.; Ochrophyta (brown), *Ecklonia* sp., *Sargassum* sp.], using liquid chromatography electrospray ionization quadrupole time-of-flight mass spectrometry (LC-ESI-QTOF-MS/MS). The total phenolic content (TPC), total flavonoid content (TFC) and total tannin content (TTC) were determined. The antioxidant potential of seaweed was assessed using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, a 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radical scavenging assay and a ferric reducing antioxidant power (FRAP) assay. Brown seaweed species showed the highest total polyphenol content, which correlated with the highest antioxidant potential. The LC-ESI-QTOF-MS/MS tentatively identified a total of 54 phenolic compounds present in the eight seaweeds. Using high-performance liquid chromatography-photodiode array (HPLC-PDA) quantification, the most abundant phenolic compound was *p*-hydroxybenzoic acid, present in *Ulva* sp. at 846.083 ± 0.02 μg/g fresh weight. The results obtained indicate the importance of seaweed as a promising source of polyphenols with antioxidant properties, consistent with the health potential of seaweed in food, pharmaceutical and nutraceutical applications.

Keywords: Seaweeds; polyphenols; antioxidant potential; LC-ESI-QTOF-MS/MS; HPLC-PDA

Table S1: Characterization of phenolic compounds in seaweeds by using LC-ESI-QTOF-MS/MS

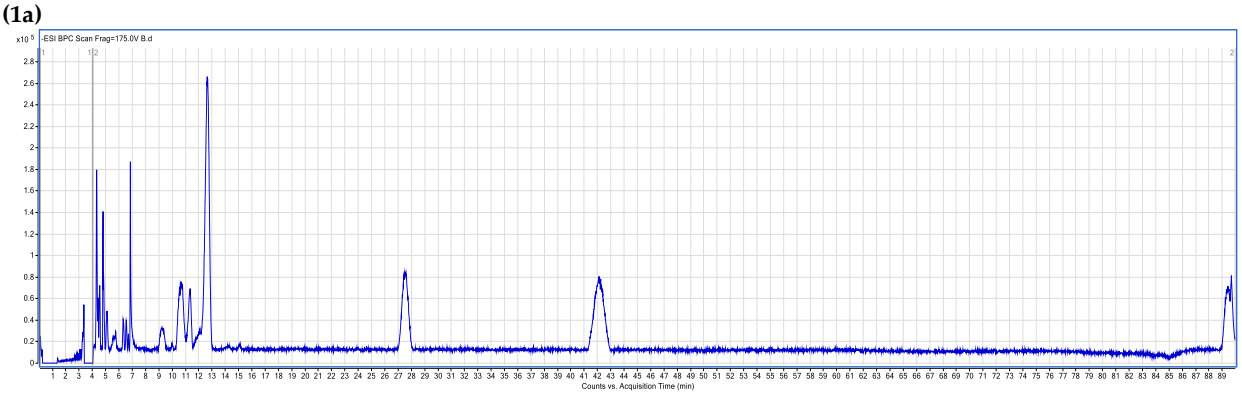
No.	Proposed compounds	Molecular Formula	RT (min)	Ionization (ESI^+/ESI^-)	Molecular Weight	Theoretical (*m*/*z*)	Observed (*m*/*z*)	Mass Error (ppm)	MS/MS Product ions	Seaweeds
Phenolic acid										
Hydroxybenzoic acids										
1	Vanillic acid 4-sulfate	$C_8H_8O_7S$	9.112	$[M-H]^-$	247.9991	246.9918	246.9925	2.83	217, 203, 167	**Sargassum* sp., *Centroceras* sp., *Ulva* sp.
2	Gallic acid	$C_7H_6O_5$	9.885	$^{**}[M-H]^-$	170.0215	169.0142	169.0138	-2.37	125	*Centroceras* sp.
3	4-Hydroxybenzoic acid 4-*O*-glucoside	$C_{13}H_{16}O_8$	11.515	$[M-H]^-$	300.0845	299.0772	299.0778	2.01	255, 137	*Sargassum* sp.
4	Protocatechuic acid 4-*O*-glucoside	$C_{13}H_{16}O_9$	13.546	$^{**}[M-H]^-$	316.0794	315.0721	315.0719	-0.63	153	**Centroceras* sp., *Grateloupia* sp.
5	*p*-Hydroxybenzoic acid	$C_7H_6O_3$	27.516	$[M-H]^-$	138.0317	137.0244	137.0240	-2.91	93	**Caulerpa* sp., *Ulva* sp., *Centroceras* sp.
6	Ellagic acid glucoside	$C_{20}H_{16}O_{13}$	38.451	$[M-H]^-$	464.0591	463.0518	463.0518	0.01	301	*Ecklonia* sp.
Hydroxycinnamic acids										
7	3-Sinapoylquinic acid	$C_{18}H_{22}O_{10}$	7.005	$^{**}[M-H]^-$	398.1213	397.1140	397.1144	1.01	223, 179	**Centroceras* sp., *Ecklonia* sp.
8	Cinnamoyl glucose	$C_{15}H_{18}O_7$	8.861	$^{**}[M-H]^-$	310.1053	309.098	309.0992	3.88	147, 131, 103	**Codium* sp., *Ulva* sp.
9	Caffeoyl glucose	$C_{15}H_{18}O_9$	10.983	$^{**}[M-H]^-$	342.0951	341.0878	341.0882	1.17	179, 161	**Ecklonia* sp., *Centroceras* sp.
10	Caffeic acid 3-*O*-glucuronide	$C_{15}H_{16}O_{10}$	14.259	$^{**}[M-H]^-$	356.0743	355.0670	355.0671	0.28	179	*Caulerpa* sp.
11	Chlorogenic acid	$C_{16}H_{18}O_9$	15.004	$^{**}[M-H]^-$	354.0951	353.0878	353.0862	-4.53	253, 190, 144	**Centroceras* sp., *Caulerpa* sp.
12	Caffeic acid	$C_9H_8O_4$	18.274	$[M-H]^-$	180.0423	179.0350	179.0350	0.01	151, 143, 133	*Caulerpa* sp.
13	Caffeic acid 4-sulfate	$C_9H_8O_7S$	18.291	$[M-H]^-$	259.9991	258.9918	258.9929	4.25	215, 179, 135	*Caulerpa* sp.
14	Caffeoyl tartaric acid	$C_{13}H_{12}O_9$	24.061	$^{**}[M-H]^-$	312.0481	311.0408	311.0403	-1.61	161	**Grateloupia* sp., *Centroceras* sp.
15	Isoferulic acid 3-sulfate	$C_{10}H_{10}O_7S$	24.520	$^{**}[M-H]^-$	274.0147	273.0074	273.0086	4.4	193, 149	*Caulerpa* sp.
16	Sinapic acid	$C_{11}H_{12}O_5$	25.852	$^{**}[M-H]^-$	224.0685	223.0612	223.0621	4.03	205, 179, 163	**Ulva* sp., *Caulerpa* sp., *Grateloupia* sp.
17	Ferulic acid	$C_{10}H_{10}O_4$	32.604	$[M-H]^-$	194.0579	193.0506	193.0513	3.63	178, 149, 134	*Caulerpa* sp.
18	Coumaric acid	$C_9H_8O_3$	33.797	$^{**}[M-H]^-$	164.0473	163.0400	163.0406	3.68	119	**Ulva* sp., *Ecklonia* sp.
19	Sinapine	$C_{16}H_{24}NO_5$	88.066	$[M+H]^+$	310.1652	310.1654	310.1646	-2.58	251, 207, 175	*Codium* sp.
Hydroxyphenylpentanoic acids										
20	5-(3',5'-dihydroxyphenyl)-γ-valerolactone 3-*O*-glucuronide	$C_{17}H_{20}O_{10}$	14.855	$^{**}[M-H]^-$	384.1056	383.0983	383.1001	4.70	221, 206, 191	**Ecklonia* sp., *Codium* sp.
21	5-(3',4'-dihydroxyphenyl)-valeric acid	$C_{11}H_{14}O_4$	51.563	$^{**}[M-H]^-$	210.0892	209.0819	209.0821	0.96	165, 150	*Caulerpa* sp.

Hydroxyphenylacetic acids										
22	2-Hydroxy-2-phenylacetic acid	$C_8H_8O_3$	6.18	**$[M+H]^+$	152.0473	153.0546	153.055	2.61	125	*_Centroceras_ sp., _Caulerpa_ sp., _Sargassum_ sp.
Flavonoids										
Anthocyanins										
23	Delphinidin 3-*O*-sambubioside	$C_{26}H_{29}O_{16}$	9.327	$[M+H]^+$	597.1464	597.1456	597.1473	2.85	303, 257, 229	_Grateloupia_ sp.
24	Isopeonidin 3-*O*-arabinoside	$C_{21}H_{21}O_{10}$	41.658	$[M+H]^+$	433.1134	433.1135	433.1136	0.23	271, 253, 243	_Centroceras_ sp.
25	Malvidin 3-*O*-glucoside	$C_{23}H_{25}O_{12}$	54.152	$[M+H]^+$	493.1343	493.1346	493.1343	-0.61	331	_Centroceras_ sp.
Flavanols										
26	Gallocatechin	$C_{15}H_{14}O_7$	7.604	**$[M-H]^-$	306.0740	305.0667	305.0668	0.33	261, 219	*_Caulerpa_ sp., _Ulva_ sp., _Dasya_ sp., _Ecklonia_ sp., _Sargassum_ sp.
27	3'-*O*-Methylcatechin	$C_{16}H_{16}O_6$	17.857	**$[M-H]^-$	304.0947	303.0874	303.0886	3.96	271, 163	_Grateloupia_ sp.
28	Catechin (isomer)	$C_{15}H_{14}O_6$	45.118	$[M-H]^-$	290.0790	289.0717	289.0731	4.84	245, 205, 179	_Caulerpa_ sp.
Flavonols										
29	Quercetin 3-*O*-(6″-malonyl-glucoside)	$C_{24}H_{22}O_{15}$	9.902	$[M-H]^-$	550.0959	549.0886	549.0887	0.18	463, 301, 161	*Centroceras sp., Caulerpa sp.
30	5,3',4'-Trihydroxy-3-methoxy-6:7-methylenedioxyflavone 4'-*O*-glucuronide	$C_{23}H_{20}O_{14}$	33.878	$[M-H]^-$	520.0853	519.0780	519.0779	-0.19	343	_Ecklonia_ sp.
31	3,7-Dimethylquercetin	$C_{17}H_{14}O_7$	80.642	$[M-H]^-$	330.0740	329.0667	329.0674	2.13	314, 299, 271	_Centroceras_ sp.
Flavones										
32	Rhoifolin	$C_{27}H_{30}O_{14}$	44.036	**$[M-H]^-$	578.1636	577.1563	577.1588	4.33	413, 269	_Centroceras_ sp.
Isoflavonoids										
33	Sativanone	$C_{17}H_{16}O_5$	4.240	$[M-H]^-$	300.0998	299.0925	299.0918	-2.34	284, 269, 225	_Ecklonia_ sp.
34	Glycitein 7-*O*-glucuronide	$C_{22}H_{20}O_{11}$	4.454	**$[M-H]^-$	460.1006	459.0933	459.0923	-2.18	283, 268, 117	_Centroceras_ sp.
35	3',4',5,7-Tetrahydroxyisoflavanone	$C_{15}H_{12}O_6$	4.640	**$[M-H]^-$	288.0634	287.0561	287.0556	-1.74	269, 259	*_Caulerpa_ sp., _Grateloupia_ sp., _Centroceras_ sp.
36	3'-*O*-Methylequol	$C_{16}H_{16}O_4$	4.803	**$[M-H]^-$	272.1049	271.0976	271.0972	-1.48	147, 123, 121	*_Ecklonia_ sp., _Grateloupia_ sp.
37	Dalbergin	$C_{16}H_{12}O_4$	9.344	**$[M-H]^-$	268.0736	267.0663	267.0666	1.12	252, 224, 180	*_Grateloupia_ sp., _Centroceras_ sp.
38	Dihydrobiochanin A	$C_{16}H_{14}O_5$	80.715	**$[M-H]^-$	286.0841	285.0768	285.0771	1.05	270	*_Codium_ sp., _Centroceras_ sp.
39	3'-Hydroxydaidzein	$C_{15}H_{10}O_5$	86.956	$[M-H]^-$	270.0528	269.0455	269.0457	0.74	151, 117, 107	*_Grateloupia_ sp., _Centroceras_ sp., _Caulerpa_ sp., _Ecklonia_ sp.
Other polyphenols										
Hydroxybenzaldehydes										

40	*p*-Hydroxybenzaldehyde	$C_7H_6O_2$	15.921	$[M-H]^-$	122.0368	121.0295	121.0295	0.01	92, 77	**Dasya* sp., *Ecklonia* sp., *Codium* sp.
Hydroxycoumarins										
41	Urolithin A	$C_{13}H_8O_4$	4.64	$[M-H]^-$	228.0423	227.0350	227.0341	-3.96	198, 182	*Grateloupia* sp.
42	Scopoletin	$C_{10}H_8O_4$	84.705	**$[M-H]^-$	192.0423	191.0350	191.0352	1.05	176, 147	**Codium* sp., *Grateloupia* sp., *Sargassum* sp.
Phenolic terpenes										
43	Rosmanol	$C_{20}H_{26}O_5$	24.965	$[M+H]^+$	346.1780	347.1853	347.1843	-2.88	301, 231	**Dasya* sp., *Ulva* sp., *Grateloupia* sp., *Ecklonia* sp., *Codium* sp.
44	Carnosol	$C_{20}H_{26}O_4$	85.931	**$[M-H]^-$	330.1831	329.1758	329.1747	-3.34	287, 286, 285	**Codium* sp., *Caulerpa* sp.
45	Carnosic acid	$C_{20}H_{28}O_4$	86.958	**$[M-H]^-$	332.1988	331.1915	331.1912	-0.91	287, 269	**Ecklonia* sp., *Dasya* sp., *Codium* sp., *Sargassum* sp.
Tyrosols										
46	Hydroxytyrosol 4-*O*-glucoside	$C_{14}H_{20}O_8$	36.653	**$[M-H]^-$	316.1158	315.1085	315.1091	1.90	153, 123	**Centroceras* sp., *Dasya* sp., *Grateloupia* sp., *Sargassum* sp.
47	3,4-DHPEA-EDA	$C_{17}H_{20}O_6$	87.423	$[M-H]^-$	320.1260	319.1187	319.1200	4.07	301, 275, 195	*Caulerpa* sp.
Other polyphenols										
48	3,4-Dihydroxyphenylglycol	$C_8H_{10}O_4$	7.005	$[M-H]^-$	170.0579	169.0506	169.0503	-1.77	141, 139, 123	*Centroceras* sp.
49	Phloroglucinol	$C_6H_6O_3$	14.793	$[M-H]^-$	126.0317	125.0244	125.0242	-1.59	97	**Ecklonia* sp., *Sargassum* sp.
50	Isopropyl 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoate	$C_{12}H_{16}O_5$	24.882	**$[M-H]^-$	240.0998	239.0925	239.0919	-2.51	195, 155, 99	*Dasya* sp.
Lignans										
Lignans										
51	2'-Hydroxyenterolactone	$C_{18}H_{18}O_5$	7.781	$[M-H]^-$	314.1154	313.1081	313.1082	0.32	295, 283	*Grateloupia* sp.
52	Arctigenin	$C_{21}H_{24}O_6$	8.131	**$[M-H]^-$	372.1573	371.1500	371.1509	2.42	356, 312, 295	**Centroceras* sp., *Sargassum* sp.
53	Dimethylmatairesinol	$C_{22}H_{26}O_6$	83.663	$[M+H]^+$	386.1729	387.1802	387.1805	0.77	372, 369, 357, 329	**Caulerpa* sp., *Dasya* sp.
54	Deoxyschisandrin	$C_{24}H_{32}O_6$	85.152	**$[M+H]^+$	416.2199	417.2272	417.2286	3.36	402, 347, 316, 301	**Ecklonia* sp., *Codium* sp., *Sargassum* sp.

* Compound was detected in more than one seaweed samples, data presented in this table are from single asterisk sample. ** Compounds were detected in both negative $[M-H]^-$ and positive $[M+H]^+$ mode of ionization while single mode data was presented. RT stands for retention time.

(1a)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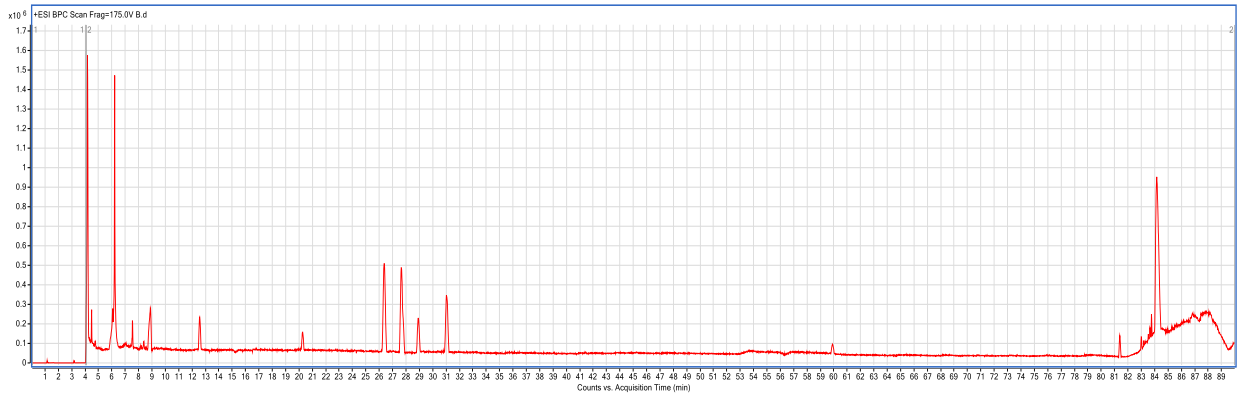
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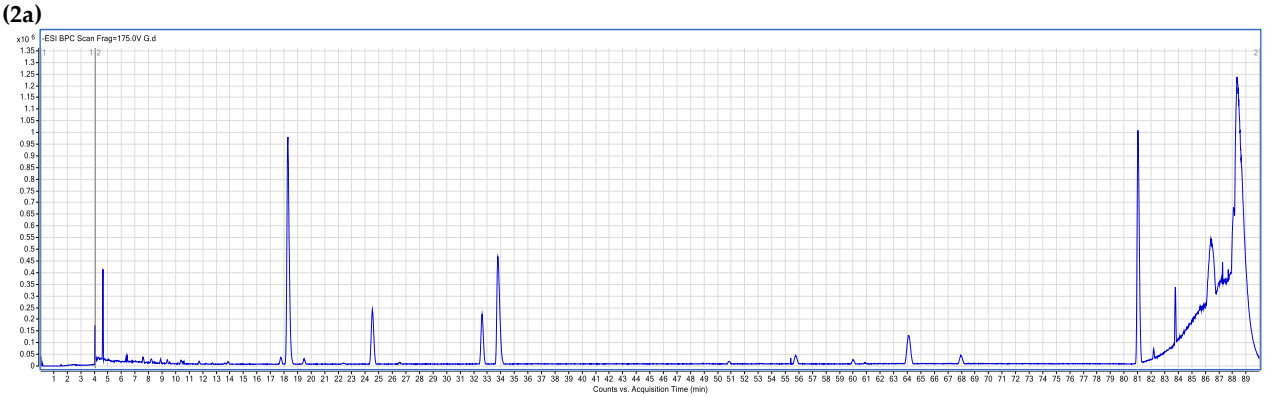
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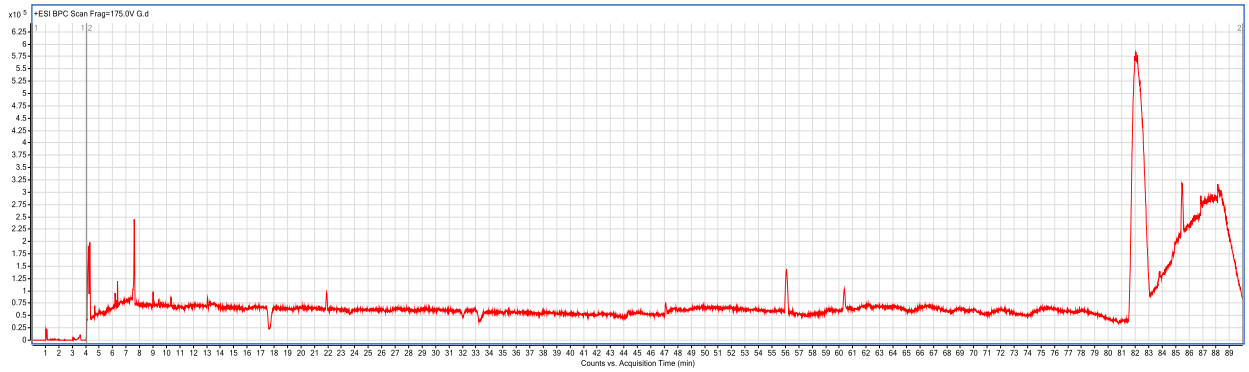
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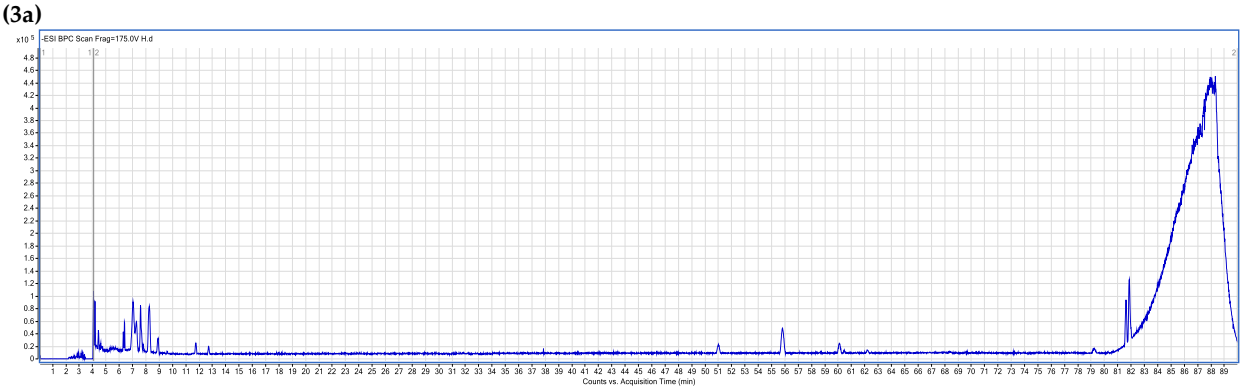
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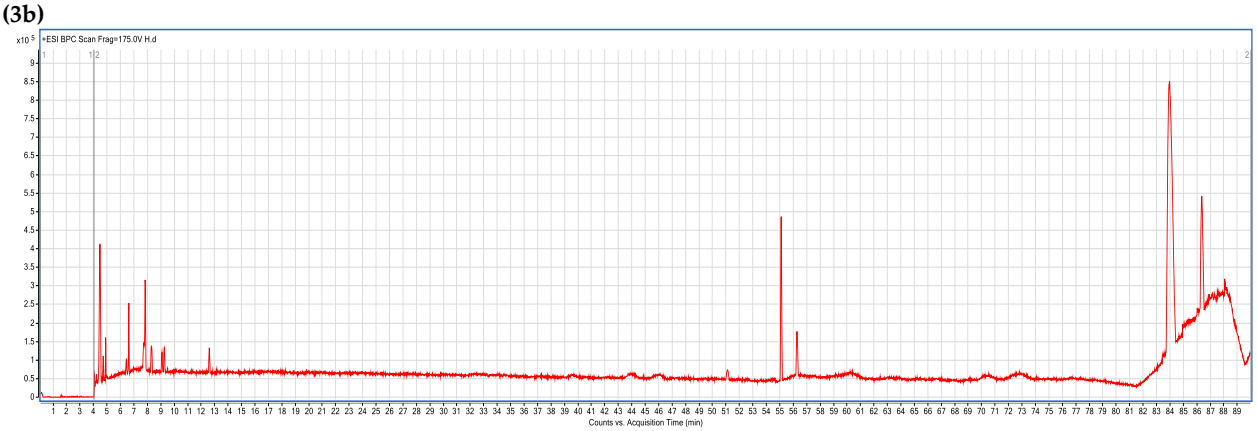
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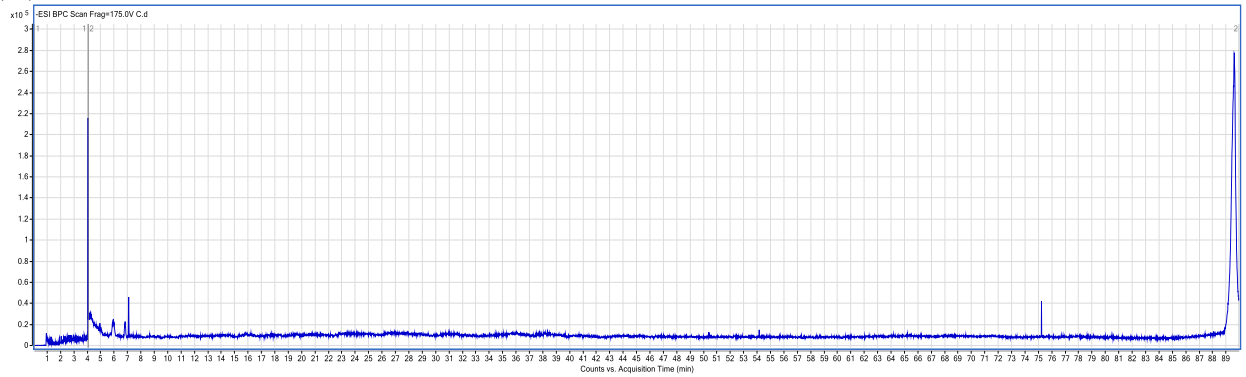
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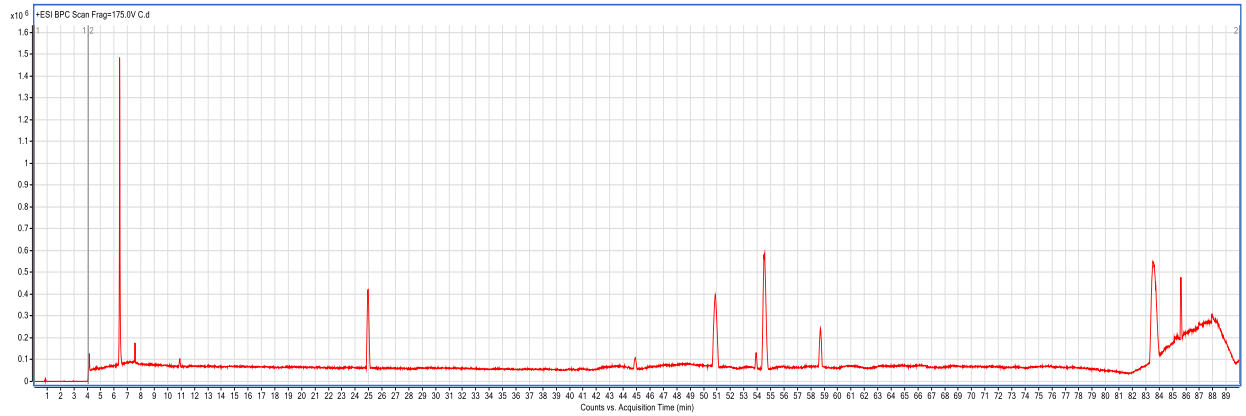
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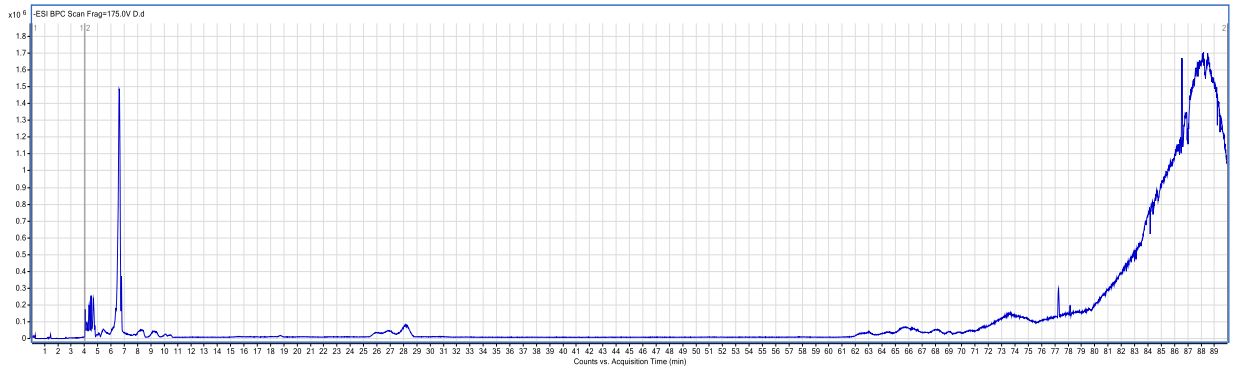
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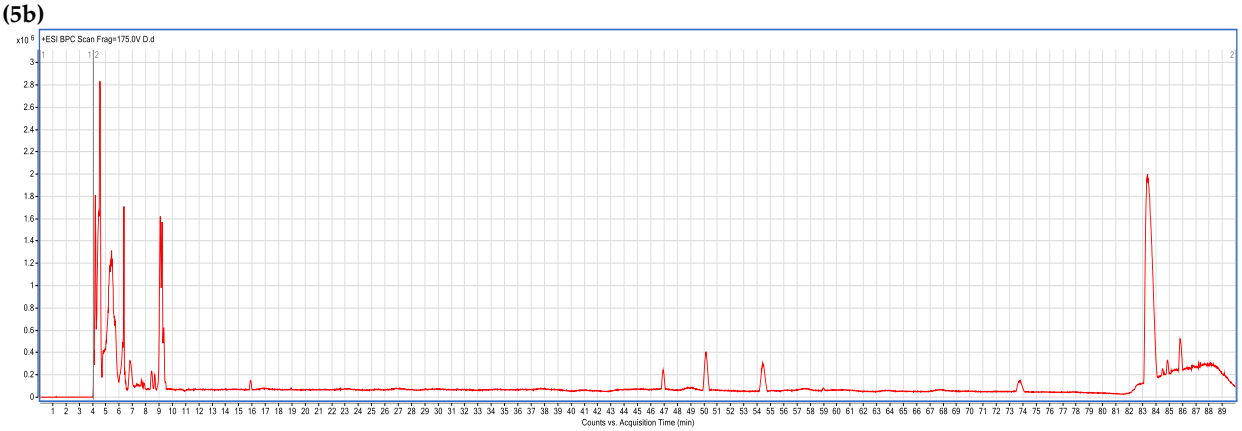
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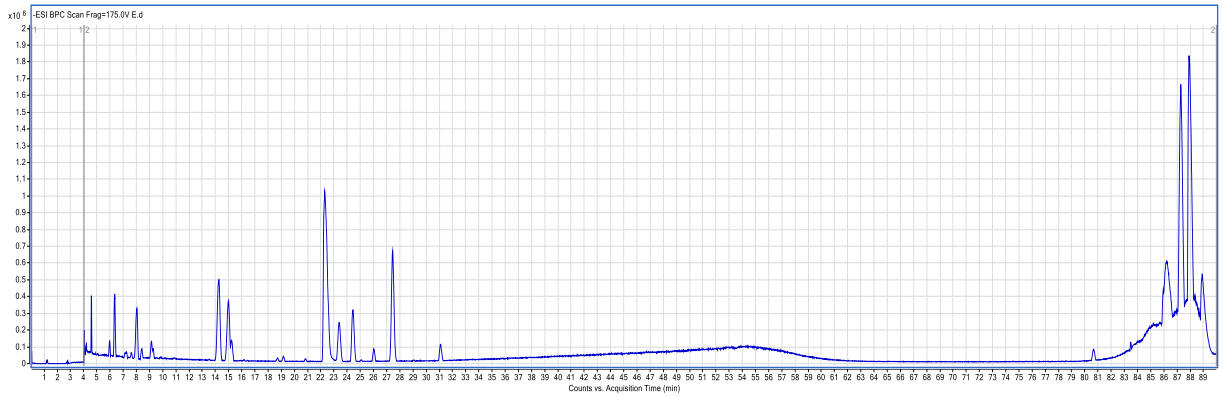
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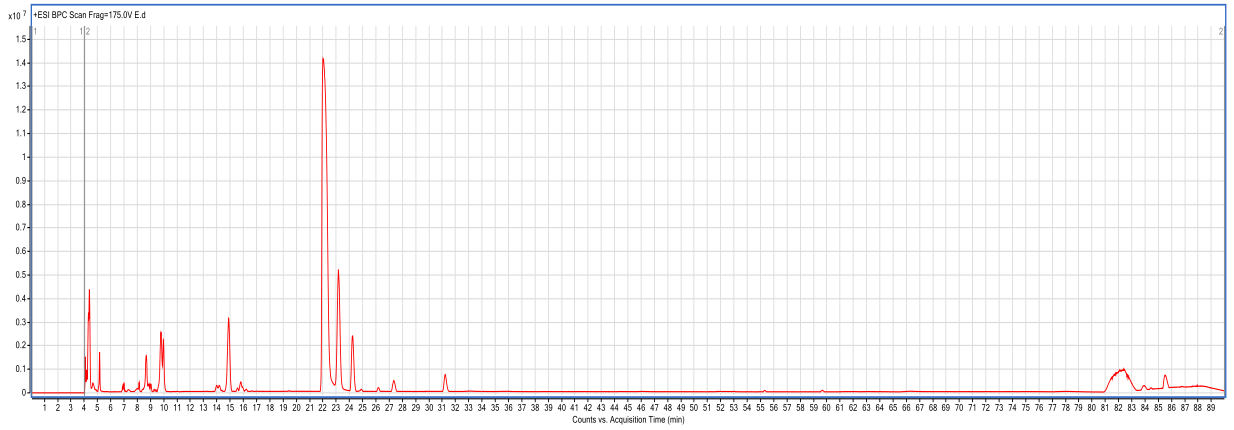
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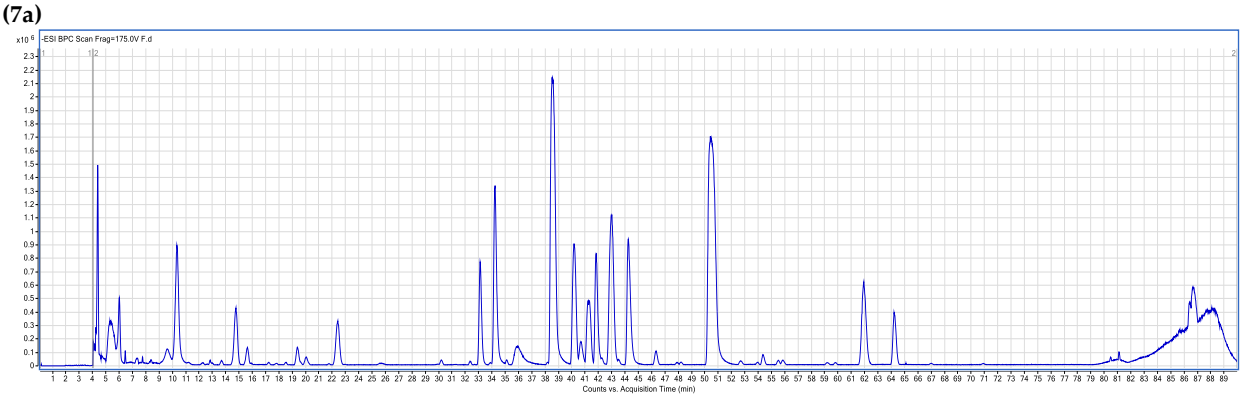
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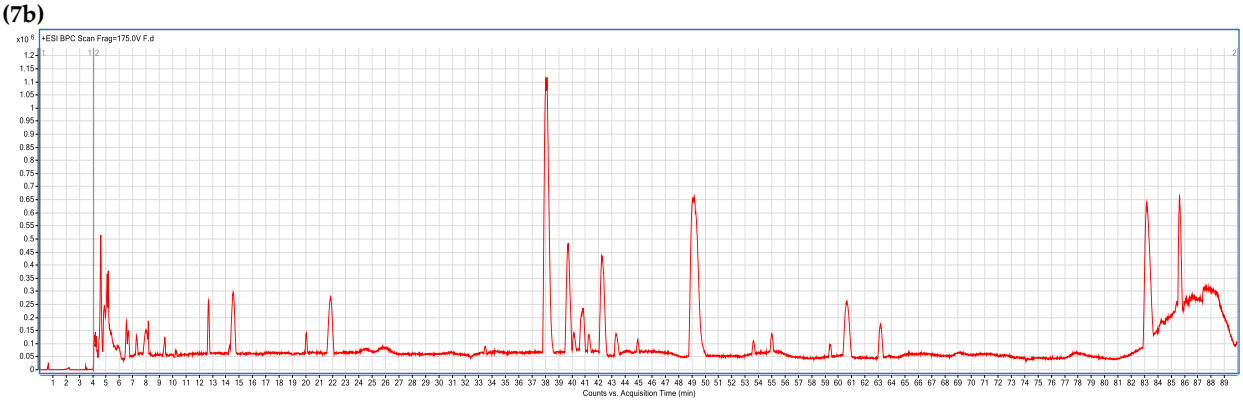
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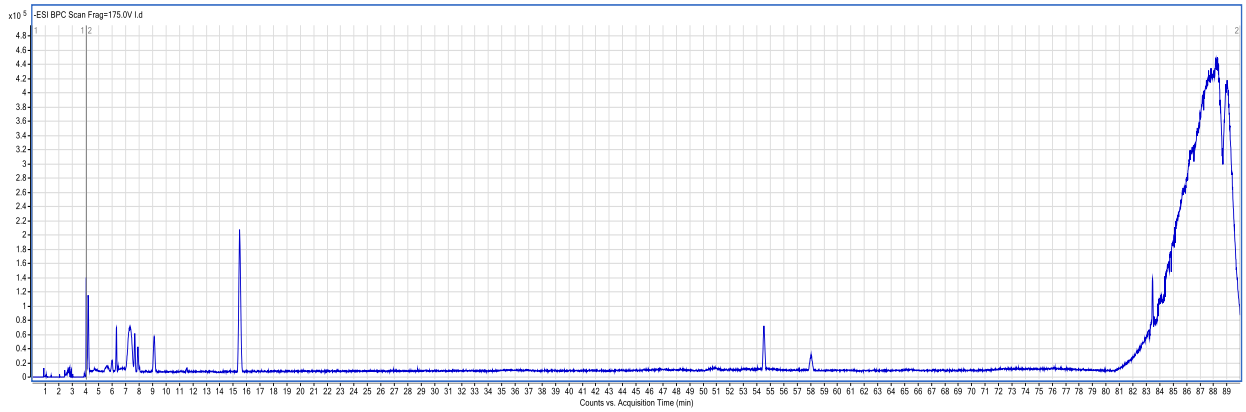
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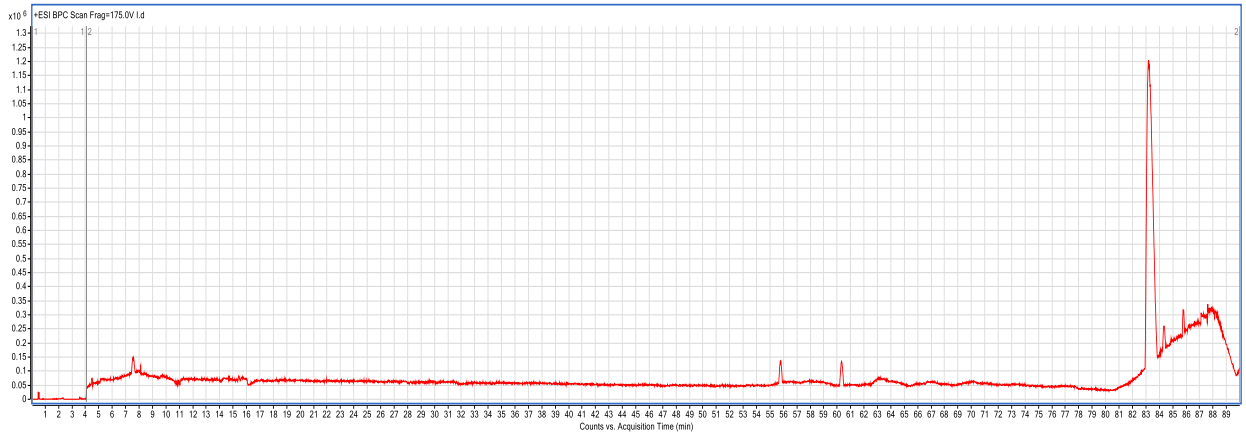
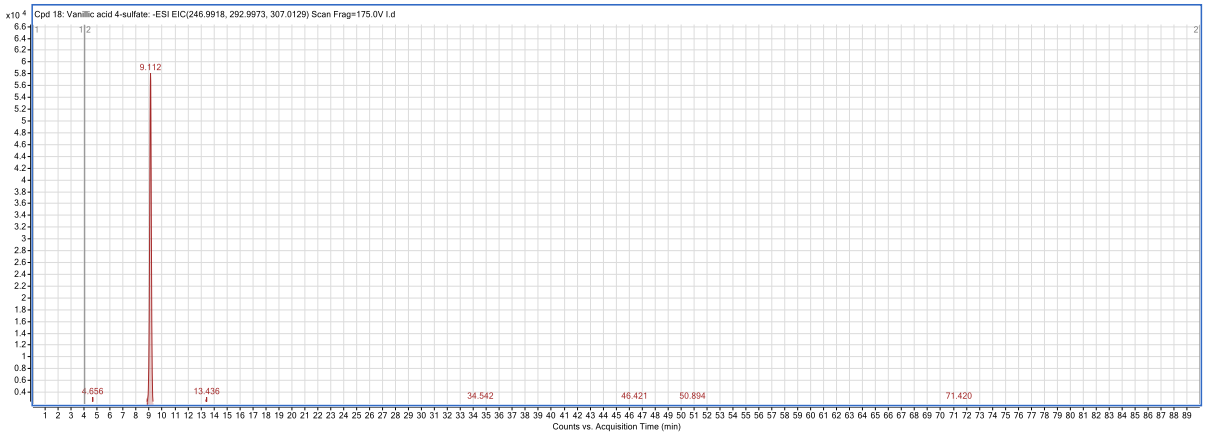
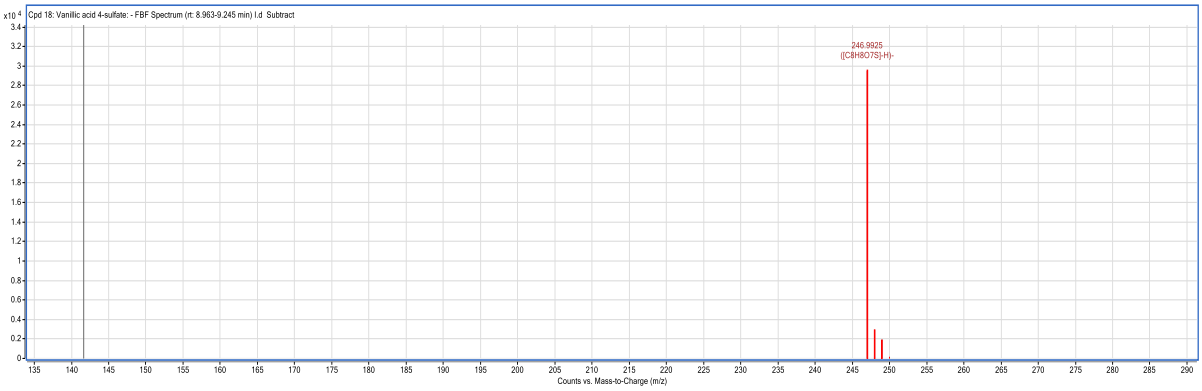

+ESI BPC Scan Frag=175.0V I.d
Counts vs. Acquisition Time (min)

Figure (S1): Base peak chromatogram (BPC) for characterization of phenolic compounds of seaweeds. (1a) *Ulva* sp. BPC in negative ionization mode; **(1b)** *Ulva* sp. BPC in positive ionization mode; **(2a)** *Caulerpa* sp. BPC in negative ionization mode; **(2b)** *Caulerpa* sp. BPC in positive ionization mode; **(3a)** *Codium* sp. BPC in negative ionization mode; **(3b)** *Codium* sp. BPC in positive ionization mode; **(4a)** *Dasya* sp. BPC in negative ionization mode; **(4b)** *Dasya* sp. BPC in positive ionization mode; **(5a)** *Grateloupia* sp. BPC in negative ionization mode; **(5b)** *Grateloupia* sp. BPC in positive ionization mode; **(6a)** *Centroceras* sp. BPC in negative ionization mode; **(6b)** *Centroceras* sp. BPC in positive ionization mode; **(7a)** *Ecklonia* sp. BPC in negative ionization mode; **(7b)** *Ecklonia* sp. BPC in positive ionization mode; **(8a)** *Sargassum* sp. BPC in negative ionization mode; **(8b)** *Sargassum* sp. BPC in positive ionization mode.

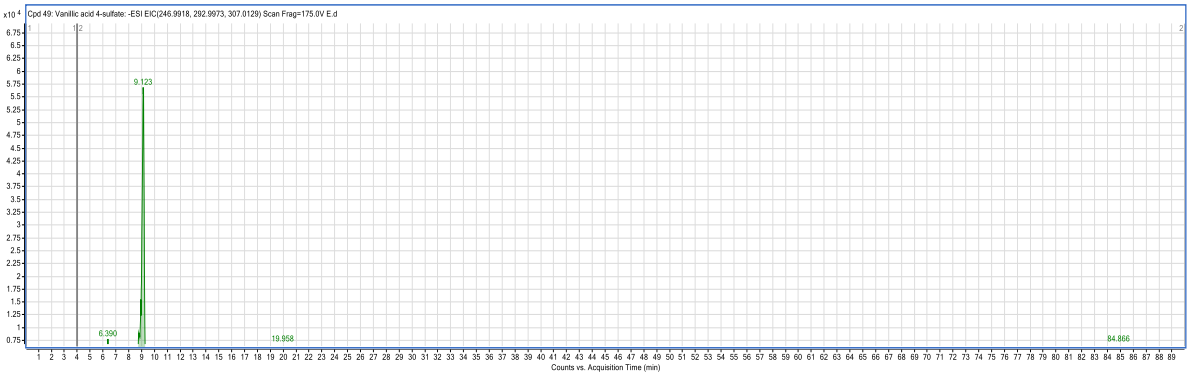
(a)


Cpd 18: Vanillic acid 4-sulfate: -ESI EIC(246.9918, 292.9973, 307.0129) Scan Frag=175.0V I.d
9.112
4.656
13.436
34.542
46.421
50.894
71.420
Counts vs. Acquisition Time (min)

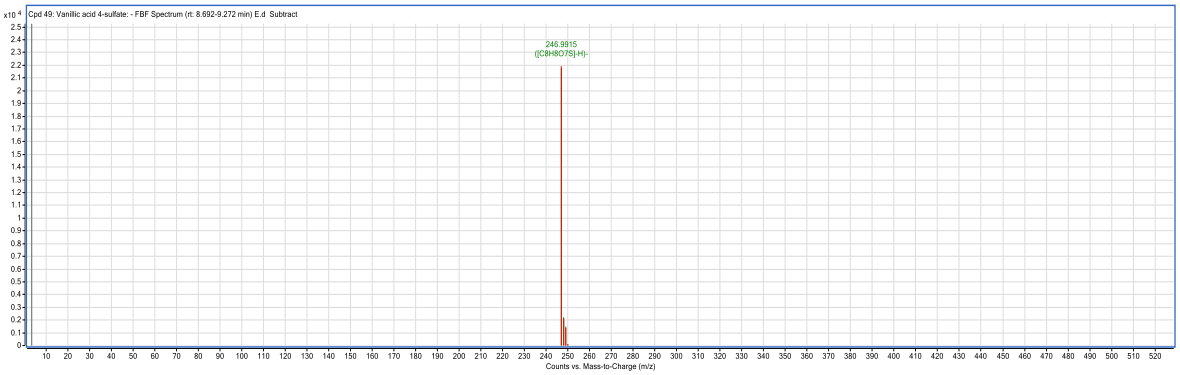
(b)


Cpd 18: Vanillic acid 4-sulfate: - FBF Spectrum (rt: 8.963-9.245 min) I.d Subtract
246.9925
([C8H8O7S]-H)-
Counts vs. Mass-to-Charge (m/z)

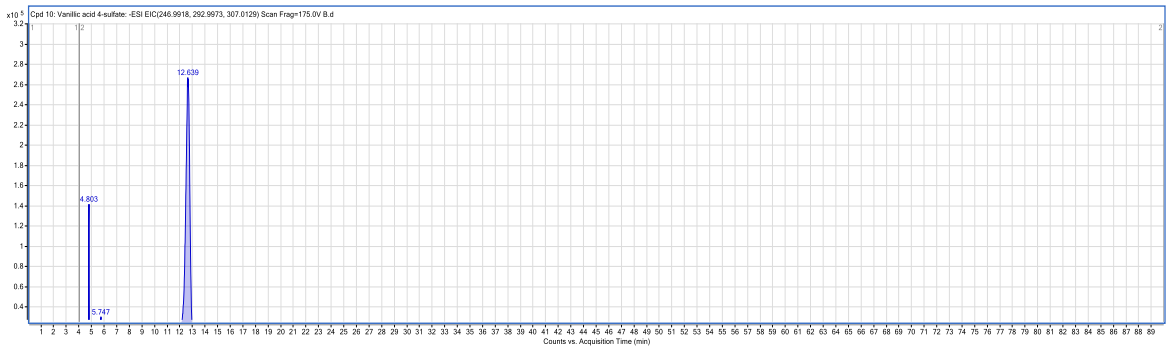
(c)


Cpd 49: Vanillic acid 4-sulfate: -ESI EIC(246.9918, 292.9973, 307.0129) Scan Frag=175.0V E.d
9.123
6.390
19.958
84.866
Counts vs. Acquisition Time (min)

(d)


Cpd 49: Vanillic acid 4-sulfate: - FBF Spectrum (rt: 8.692-9.272 min) E.d Subtract
246.9915
([C8H8O7S]-H)-
Counts vs. Mass-to-Charge (m/z)

(e)



(f)

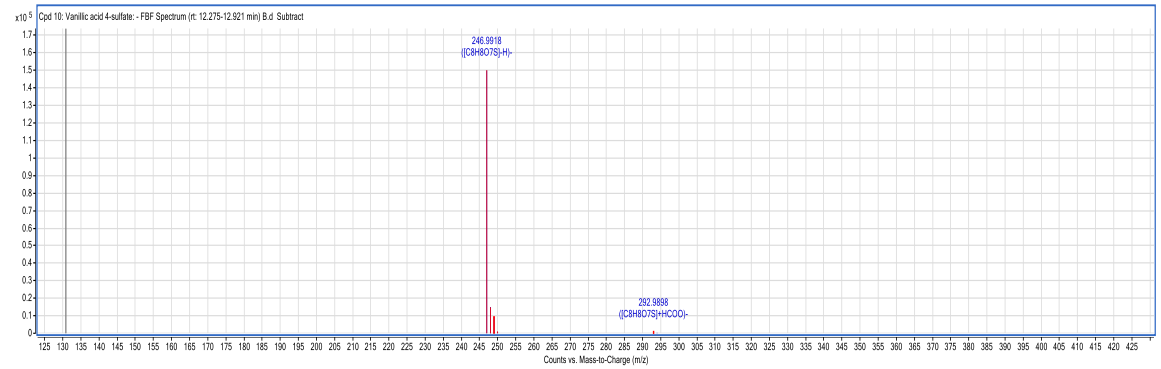


Figure (S2). Extracted ion chromatogram and mass spectrum of "Vanillic acid 4-sulfate" detected in three different seaweeds. (a) A chromatograph of vanillic acid 4-sulfate (Compound **1**, Table 1S) identified in *Sargassum* sp. in negative mode of ionization (ESI^-/$[M-H]^-$); **(b)** Mass spectra of vanillic acid 4-sulfate showing an observed *m*/*z* 246.9925 in *Sargassum* sp. **(c)** A chromatograph of vanillic acid 4-sulfate identified in *Centroceras* sp. in negative mode; **(d)** Mass spectra of vanillic acid 4-sulfate showing an observed *m*/*z* 246.9915 in *Centroceras* sp.; **(e)** A chromatograph of vanillic acid 4-sulfate identified in *Ulva* sp. in negative mode; **(f)** Mass spectra of vanillic acid 4-sulfate showing an observed *m*/*z* 246.9918 in *Ulva* sp.