

## Supporting Information

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# LC-HRMS Based Approach for Identification and Quantification Analysis of Chemical Constituents of Sea Cucumbers from Aegean Sea - Their Cytotoxic and Antiviral Potentials

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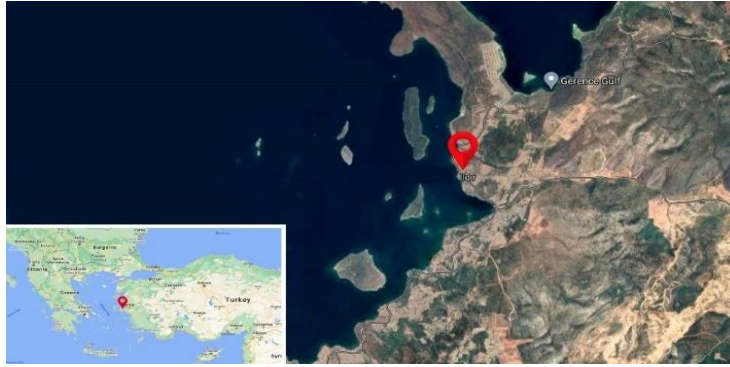
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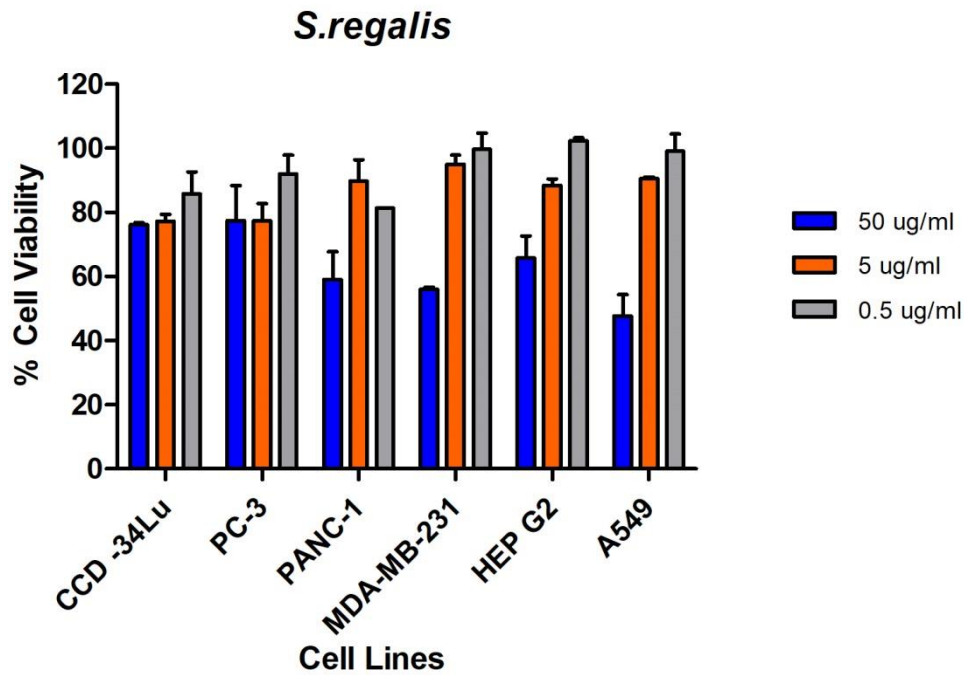
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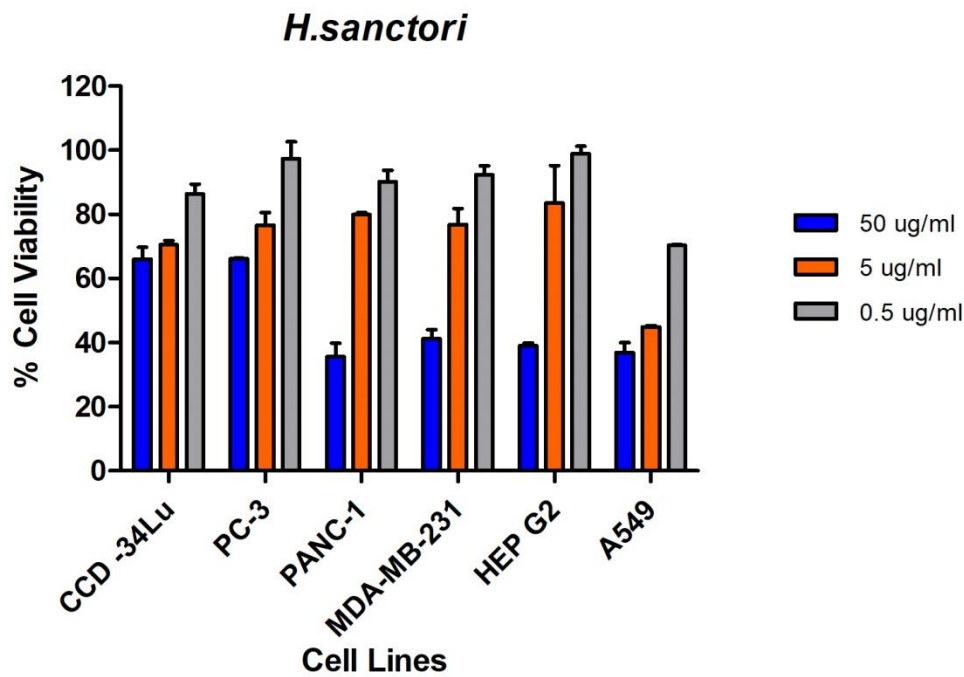
**Figure S1:** Collecting area of sea cucumbers

**Table S1:** Mass parameters and linear regression equation of compounds

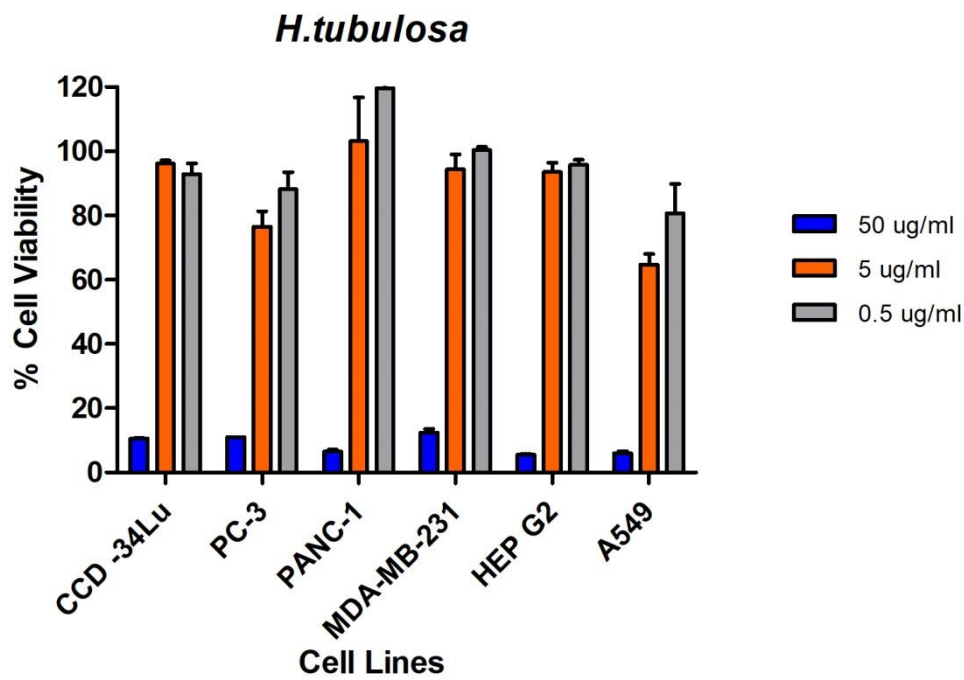
Compounds	Molecule Formula	m/z	Ionization Mode	Linear	Linear Regression Equation	LOD/LOQ	r <sup>2</sup>	Recovery
Ascorbic acid	C <sub>6</sub> H <sub>8</sub> O <sub>6</sub>	175.0248	Negative	0.5-10	y=0.00347x-0.00137	0.39/1.29	0.9988	96.2
Chlorogenic acid	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	353.0878	Negative	0.05-10	y=0.00817x+0.000163	0.02/0.06	0.9994	96.7
Fumaric acid	C <sub>4</sub> H <sub>4</sub> O <sub>4</sub>	115.0037	Negative	0.1-10	y=0.00061x-0.0000329	0.05/0.17	0.9991	97.1
Orientin	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	447.0933	Negative	0.1-10	y=0.00757x+0.000347	0.01/0.03	0.9993	96.2
Caffeic acid	C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>	179.0350	Negative	0.3-10	y=0.0304x+0.00366	0.08/0.27	0.9993	94.5
Caffeine	C <sub>8</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub>	195.0877	Positive	0.05-7	y=0.122x+0.00366	0.01/0.03	0.9987	92.9
Luteolin-7-rutinoside	C <sub>27</sub> H <sub>30</sub> O <sub>15</sub>	593.1512	Negative	0.1-10	y=0.00879x+0.000739	0.01/0.03	0.9988	93.0
Vanilic acid	C <sub>8</sub> H <sub>8</sub> O <sub>4</sub>	167.0350	Negative	0.3-10	y=0.00133x+0.0003456	0.1/0.33	0.9997	98.7
Luteolin-7-glucoside	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	447.0933	Negative	0.1-7	y=0.0162x+0.00226	0.01/0.03	0.9961	96.3
Hyperoside	C <sub>21</sub> H <sub>20</sub> O <sub>12</sub>	463.0882	Negative	0.05-10	y=0.0072x-0.00003096	0.01/0.03	0.9995	96.6
Apigenin-7-glucoside	C <sub>21</sub> H <sub>20</sub> O <sub>10</sub>	431.0984	Negative	0.3-7	y=0.0246x+0.00306	0.01/0.03	0.9962	96.0
Salicylic acid	C <sub>7</sub> H <sub>6</sub> O <sub>3</sub>	137.0244	Negative	0.3-10	y=0.0361x+0.00245	0.01/0.03	0.9982	92.9
Naringenin	C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>	271.0612	Negative	0.1-10	y=0.0281x+0.00182	0.01/0.03	0.9995	86.7
Luteolin	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	285.0405	Negative	0.1-10	y=0.117x+0.00848	0.01/0.03	0.9981	96.9
Nepetin	C <sub>16</sub> H <sub>12</sub> O <sub>7</sub>	315.0510	Negative	0.05-10	y=0.0853x+0.00269	0.01/0.03	0.9992	97.8
Apigenin	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>	269.0456	Negative	0.3-10	y=0.104x+0.0199	0.01/0.03	0.9998	81.6
Sinensetin	C <sub>20</sub> H <sub>20</sub> O <sub>7</sub>	373.1282	Positive	0.5-10	y=0.415x+0.0761	0.01/0.03	0.9967	93.3
CAPE	C <sub>17</sub> H <sub>16</sub> O <sub>4</sub>	283.0976	Negative	0.3-7	y=0.255x+0.0477	0.01/0.03	0.9964	94.4
Chrysin	C <sub>15</sub> H <sub>10</sub> O <sub>4</sub>	253.0506	Negative	0.05-7	y=0.0964x-0.0002622	0.01/0.03	0.999	87.9
Cephoside B	C <sub>65</sub> H <sub>106</sub> O <sub>32</sub>	1397.659	Negative	0.25-5	y=0.03048x-0.003199	0.08/0.26	0.9988	102.8
Dipsacoside B	C <sub>53</sub> H <sub>86</sub> O <sub>22</sub>	1075.568	Positive	0.5-10	y=0.003923x-0.0003301	0.15/0.5	0.9995	108.6
Saponin A	C <sub>47</sub> H <sub>76</sub> O <sub>18</sub> Na	952.5002	Positive	0.5-10	y=0.02322x-0.001075	0.11/0.36	0.9995	106.4
Scoposide B	C <sub>64</sub> H <sub>104</sub> O <sub>30</sub> Na	1351.654	Negative	0.1-2	y=0.08182x+0.0002178	0.02/0.08	0.9995	98.0
Saponin B	C <sub>53</sub> H <sub>87</sub> O <sub>23</sub>	1090.557	Negative	0.2-5	y=0.03348x-0.002113	0.22/0.73	0.9962	95.5
Aristatoside C	C <sub>57</sub> H <sub>92</sub> O <sub>25</sub> Na	1175.585	Negative	0.5-10	y=0.01879x-0.002058	0.13/0.44	0.9994	92.9
Tormentic acid	C <sub>30</sub> H <sub>48</sub> O <sub>5</sub>	511.3394	Positive	0.1-2	y=0.08359x+0.003066	0.03/0.11	0.9983	101.0
Balansoid A	C <sub>52</sub> H <sub>84</sub> O <sub>20</sub> Na	1027.548	Negative	0.5-10	y=0.02999x-0.002864	0.19/0.62	0.998	102.3
Macranthoside A	C <sub>47</sub> H <sub>76</sub> O <sub>17</sub>	935.4975	Positive	0.5-10	y=0.05647x-0.004836	0.09/0.29	0.9996	102.7
Dipsacus saponin A	C <sub>42</sub> H <sub>69</sub> O <sub>14</sub>	796.4615	Negative	0.5-10	y=0.06377x-0.01101	0.2/0.67	0.998	109.4
Pomolic acid	C <sub>30</sub> H <sub>48</sub> O <sub>4</sub>	511.3394	Positive	0.5-10	y=0.005607x-0.001334	0.17/0.56	0.9995	100.9



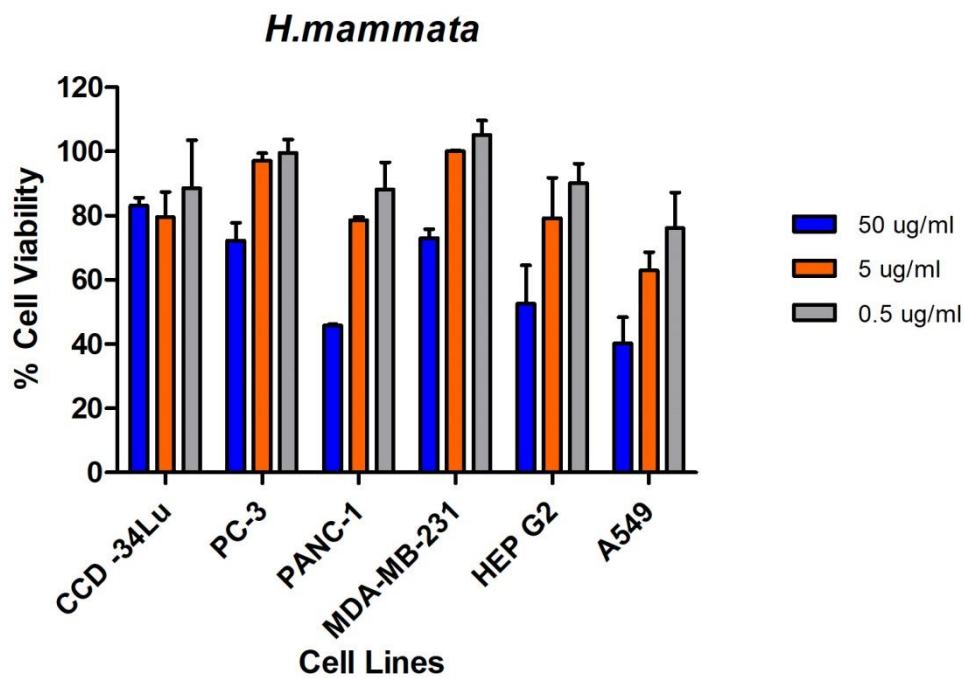
**Figure S2:** Viability of *S. regalis* against the cell lines.



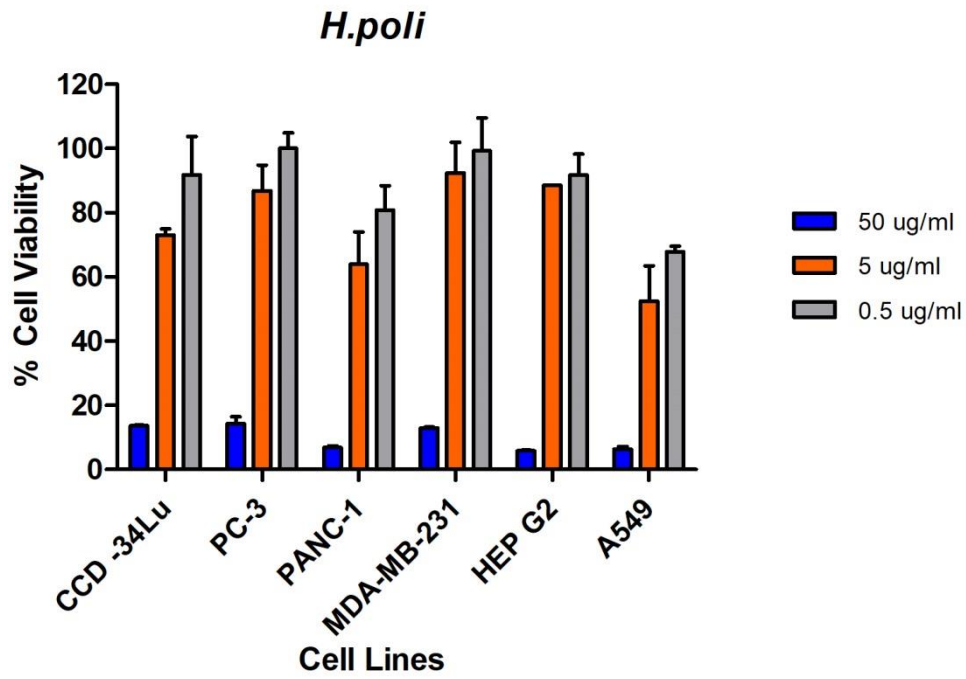
**Figure S3:** Viability of *H. sanctori* against the cell lines.



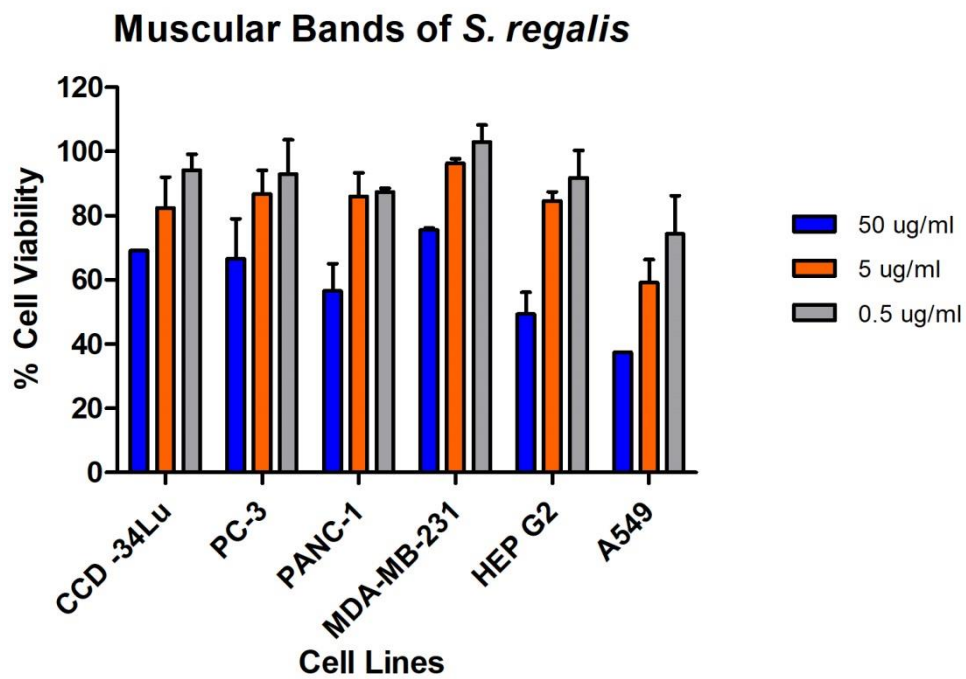
**Figure S4:** Viability of *H. tubulosa* against the cell lines



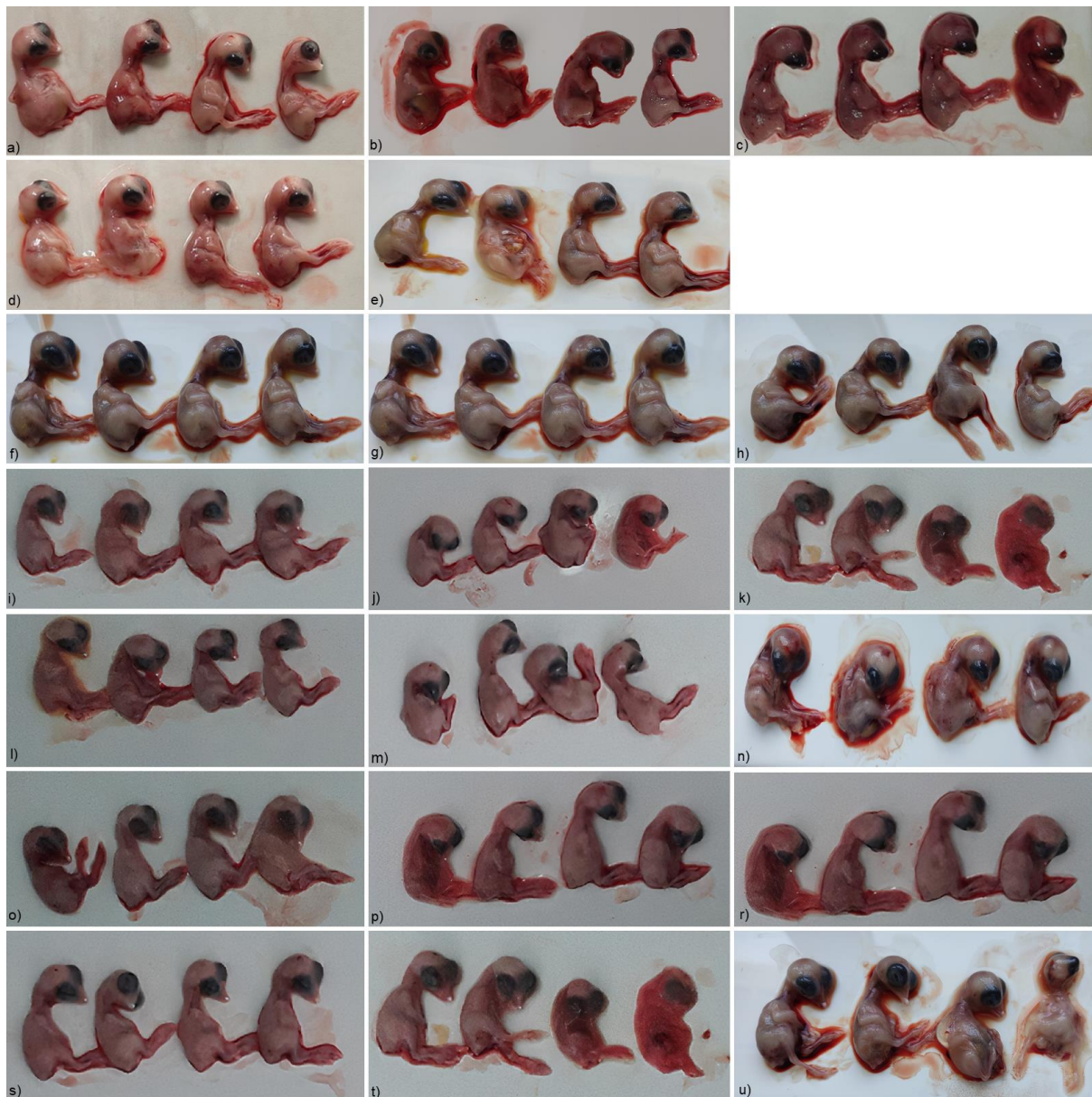
**Figure S5:** Viability of *H. mammata* against the cell lines



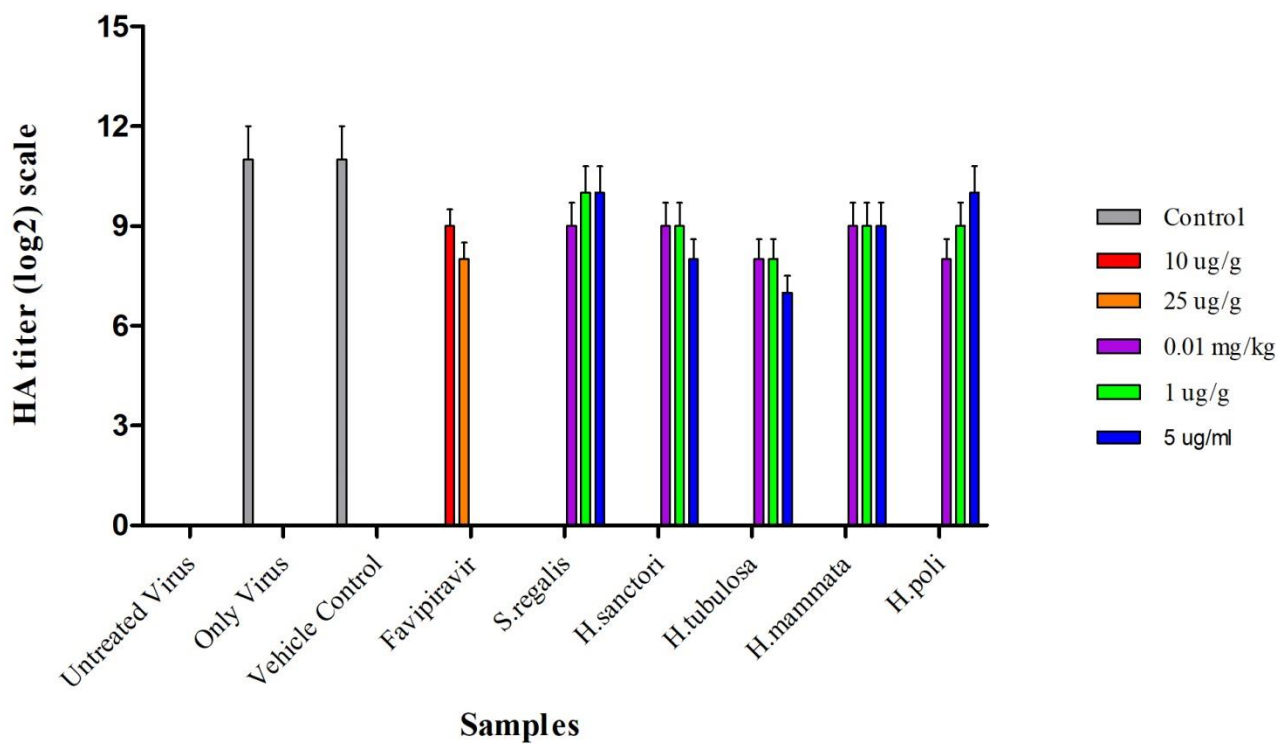
**Figure S6:** Viability of *H. poli* against the cell lines



**Figure S7:** Viability of muscular bands of *S. regalis* against the cell lines



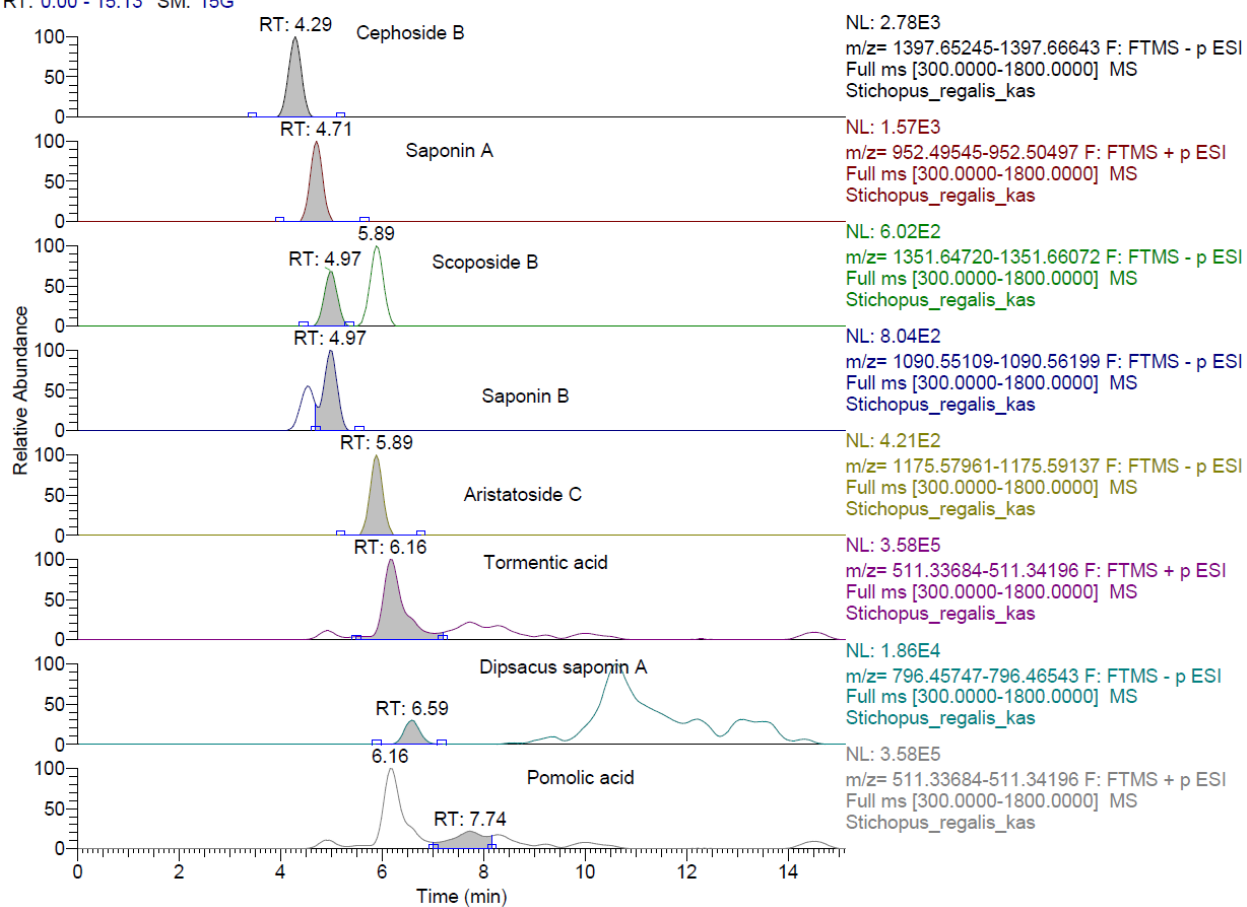
**Figure S8:** Embryos after 48h incubation with sea cucumber-IBV mixture a) Negative untreated SPF-ECE control b) Negative control, only virus c) Negative vehicle control, treated with %5 DMSO d) Positive antiviral drug control, Favipiravir 10  $\mu\text{g/g}$  e) Positive antiviral drug control, Favipiravir 25  $\mu\text{g/g}$ , f) *S.regalis* sample, 0.01  $\mu\text{g/g}$ , g) *S.regalis* sample, 1  $\mu\text{g/g}$ , h) *S.regalis* sample, 5  $\mu\text{g/g}$ , i) *H.sanctori* sample, 0.01  $\mu\text{g/g}$ , j) *H.sanctori* sample, 1  $\mu\text{g/g}$ , k) *H.sanctori* sample, 5  $\mu\text{g/g}$ , l) *H.tubulosa* sample, 0.01  $\mu\text{g/g}$ , m) *H.tubulosa* sample, 1  $\mu\text{g/g}$ , n) *H.tubulosa* sample, 5  $\mu\text{g/g}$ , o) *H.mammata* sample, 0.01  $\mu\text{g/g}$ , p) *H.mammata* sample, 1  $\mu\text{g/g}$ , r) *H.mammata* sample, 5  $\mu\text{g/g}$ , s) *H.poli* sample, 0.01  $\mu\text{g/g}$ , t) *H.poli* sample, 1  $\mu\text{g/g}$ , u) *H.poli* sample, 5  $\mu\text{g/g}$ .



**Figure S9:** HA titers of SPF-ECE after 1h incubation with IBV-sea cucumber and IBV-favipiravir mixture samples

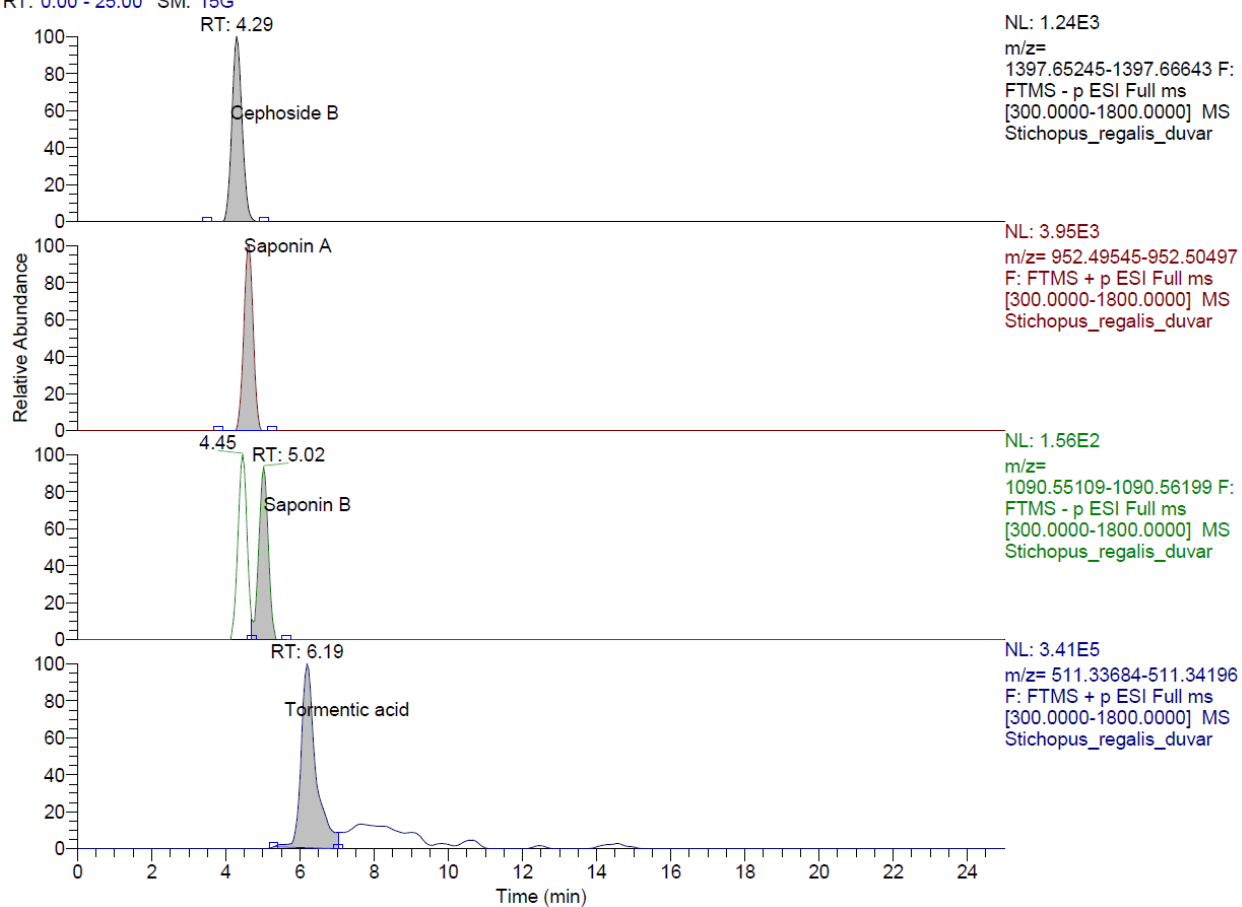


RT: 0.00 - 15.13 SM: 15G

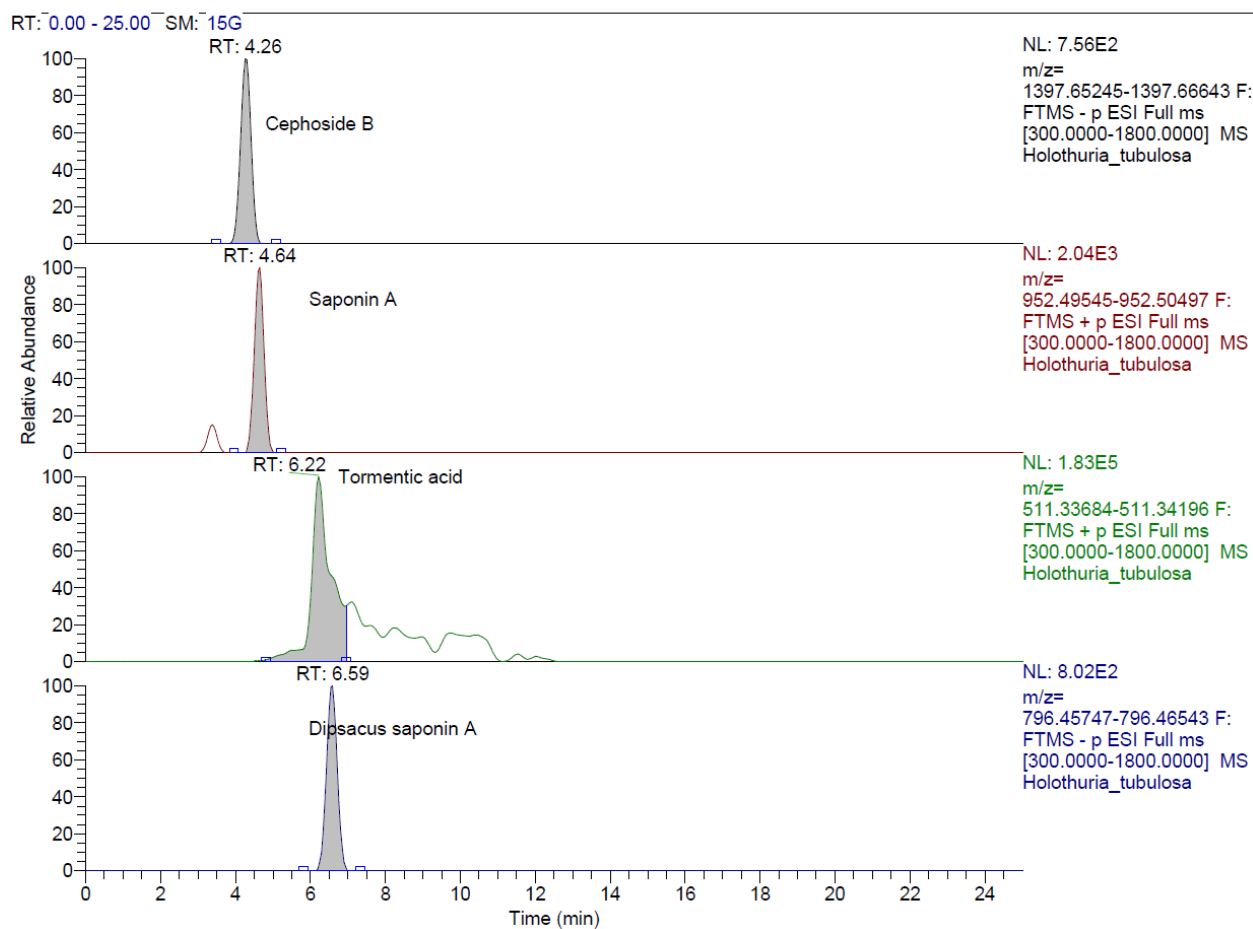


**Figure S10:** LC-HRMS chromatogram of *n*-butanol extract of *S. regalis*

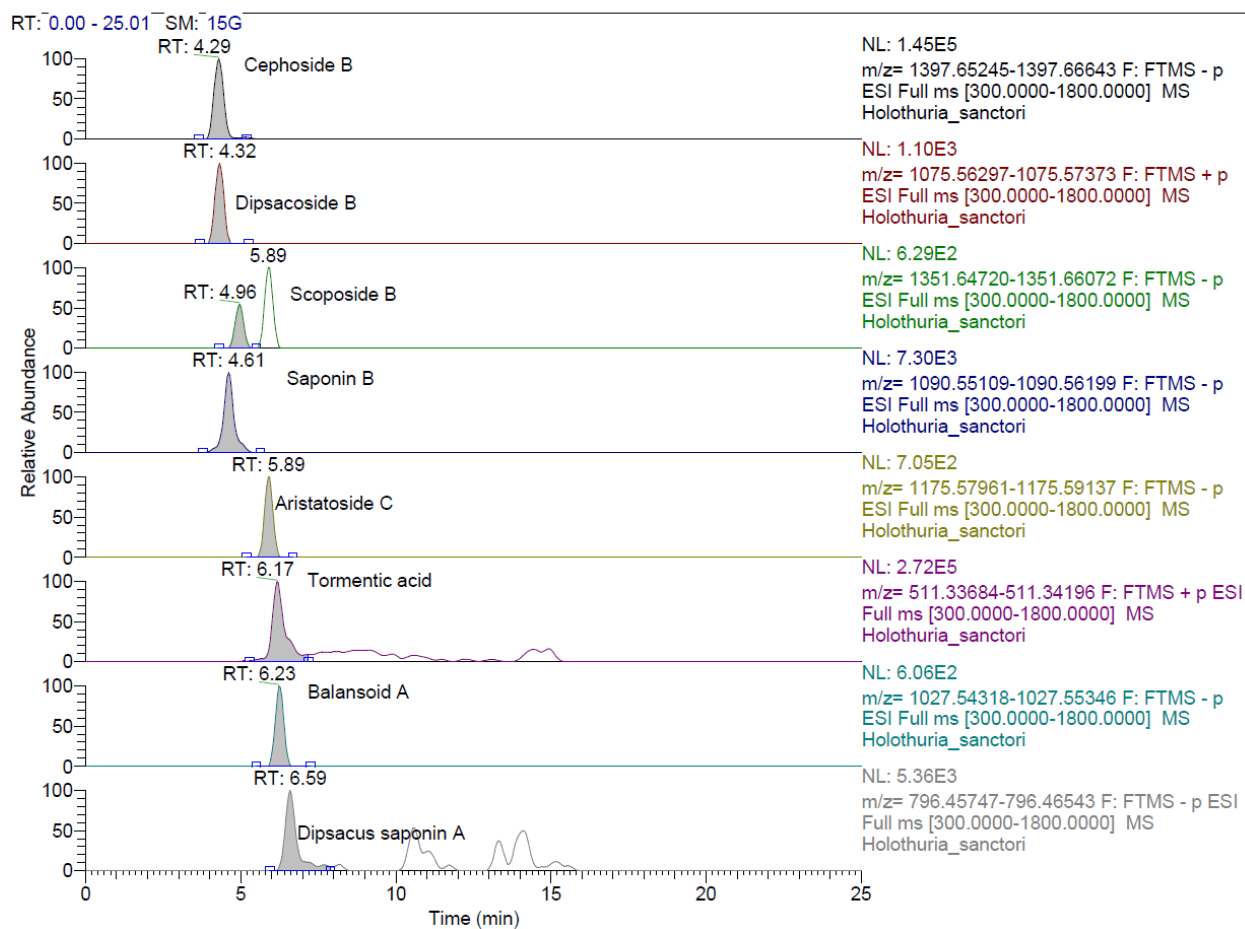
RT: 0.00 - 25.00 SM: 15G



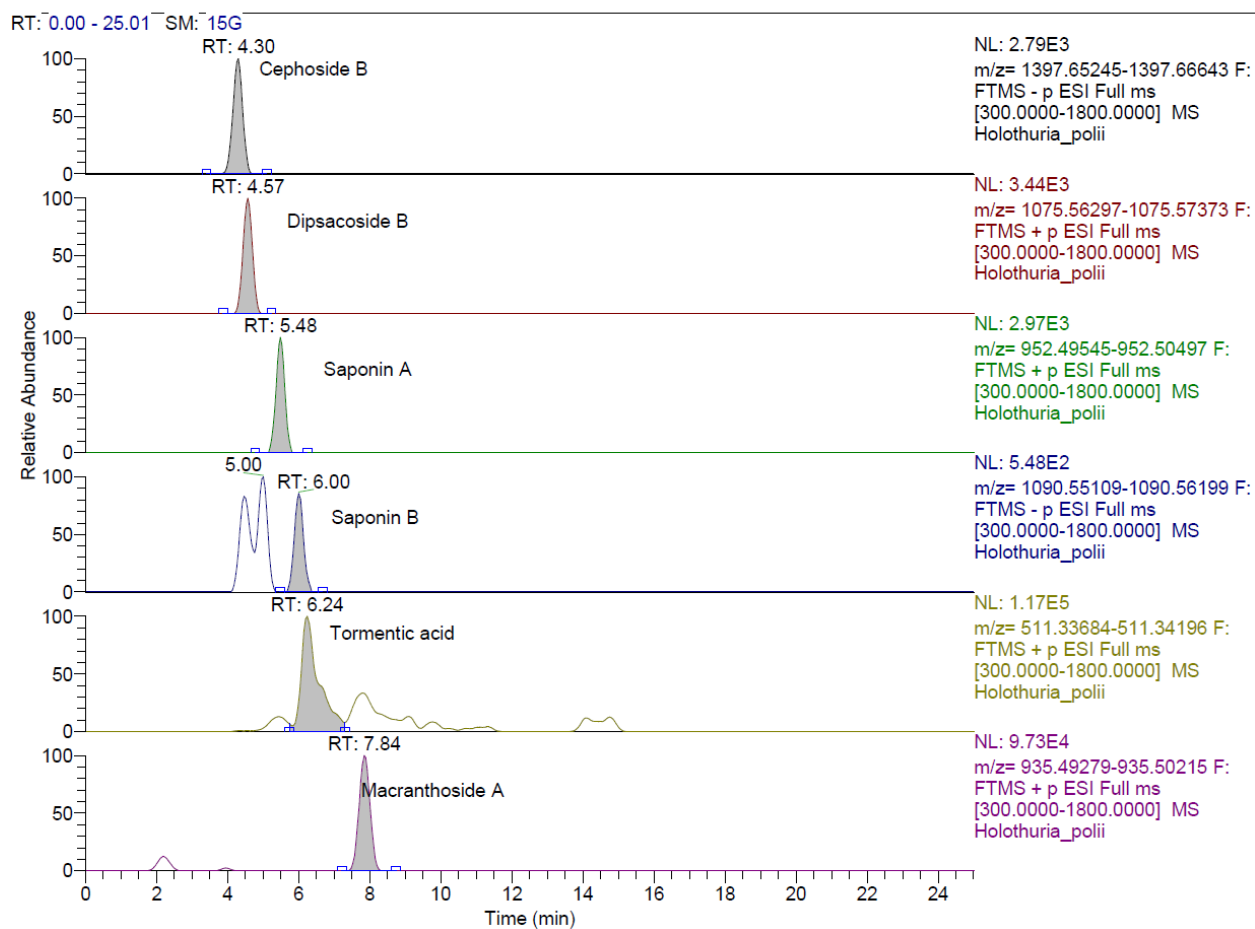
**Figure S11:** LC-HRMS chromatogram of *n*-butanol extract of muscular bands on *S. regalis*



**Figure S12:** LC-HRMS chromatogram of *n*-butanol extract of *H. tubulosa*

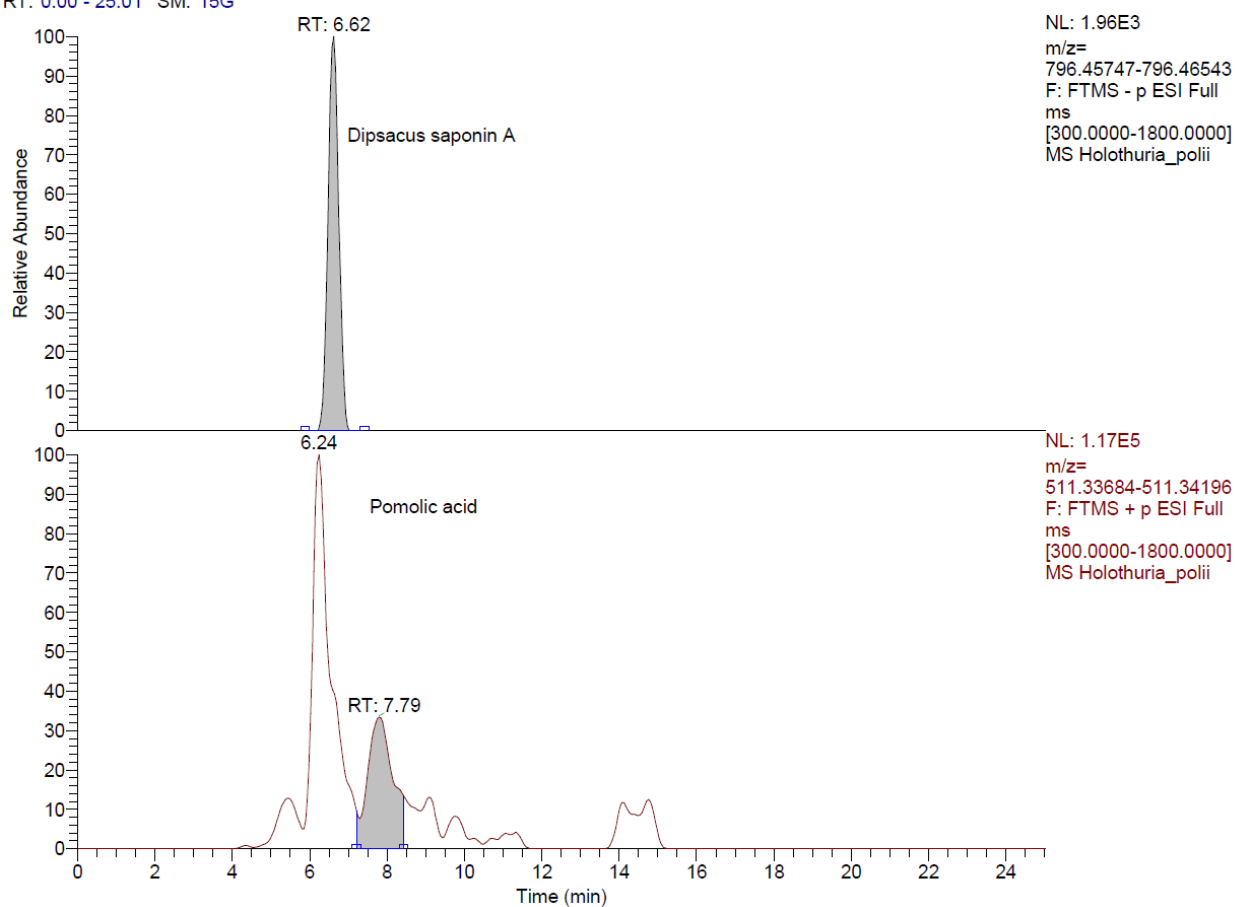


**Figure S13:** LC-HRMS chromatogram of *n*-butanol extract of *H. sanctori*



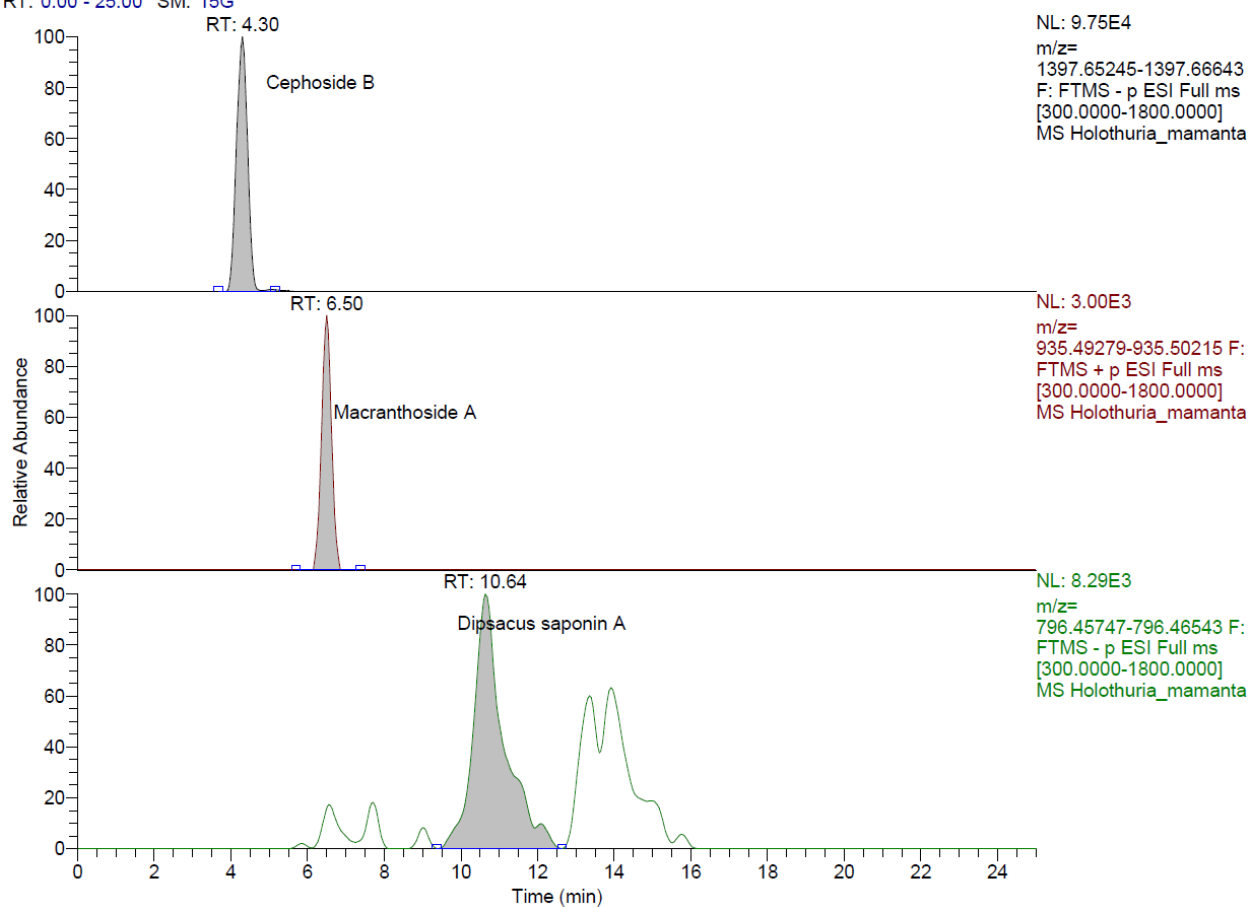
**Figure S14:** LC-HRMS chromatogram of *n*-butanol extract of *H. poli*

RT: 0.00 - 25.01 SM: 15G



**Figure S15:** LC-HRMS chromatogram of *n*-butanol extract of *H. poli*

RT: 0.00 - 25.00 SM: 15G



**Figure S16:** LC-HRMS chromatogram of *n*-butanol extract of *H. mamanta*

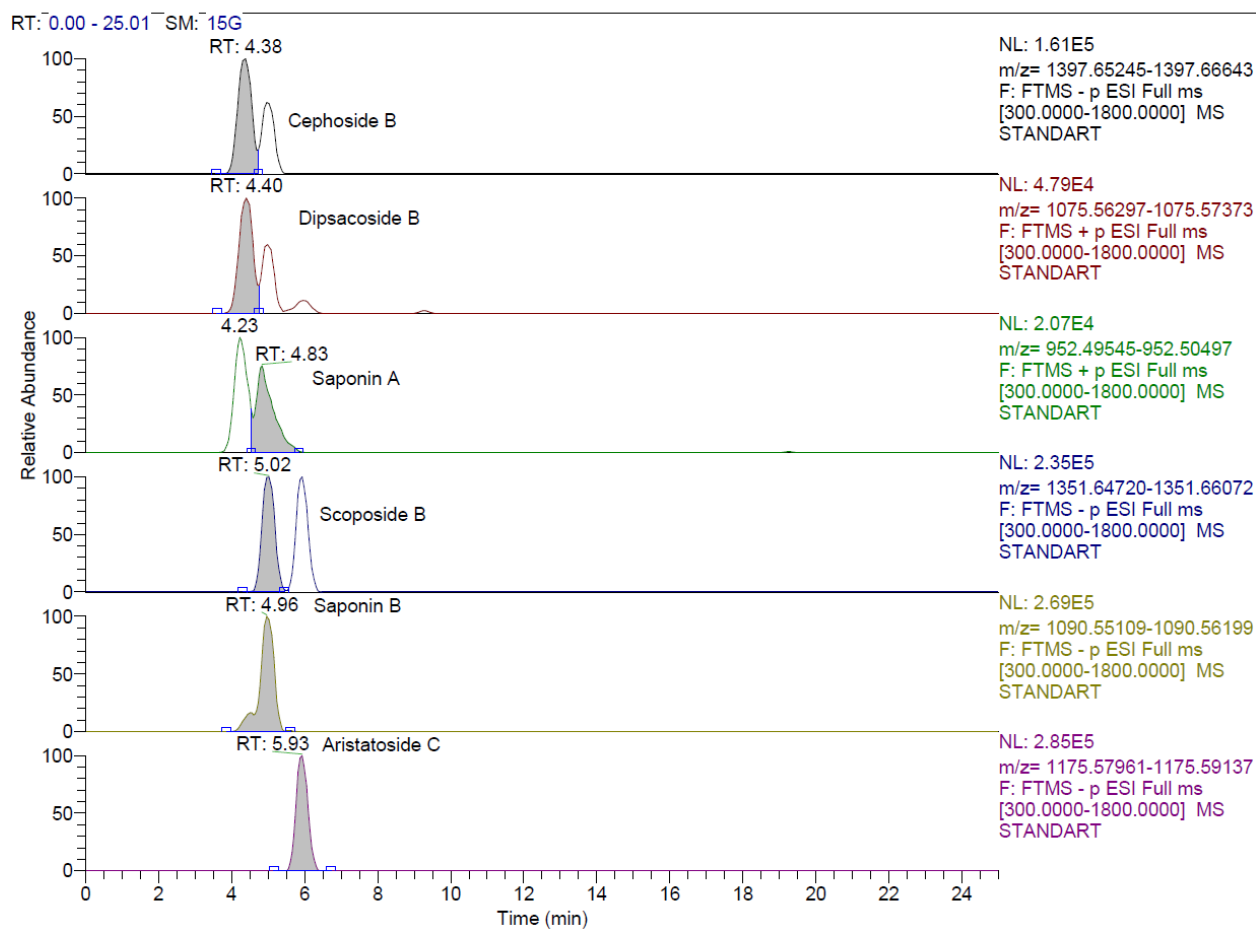


Figure S17: LC-HRMS chromatogram of standard mixture



RT: 0.00 - 25.01 SM: 7G

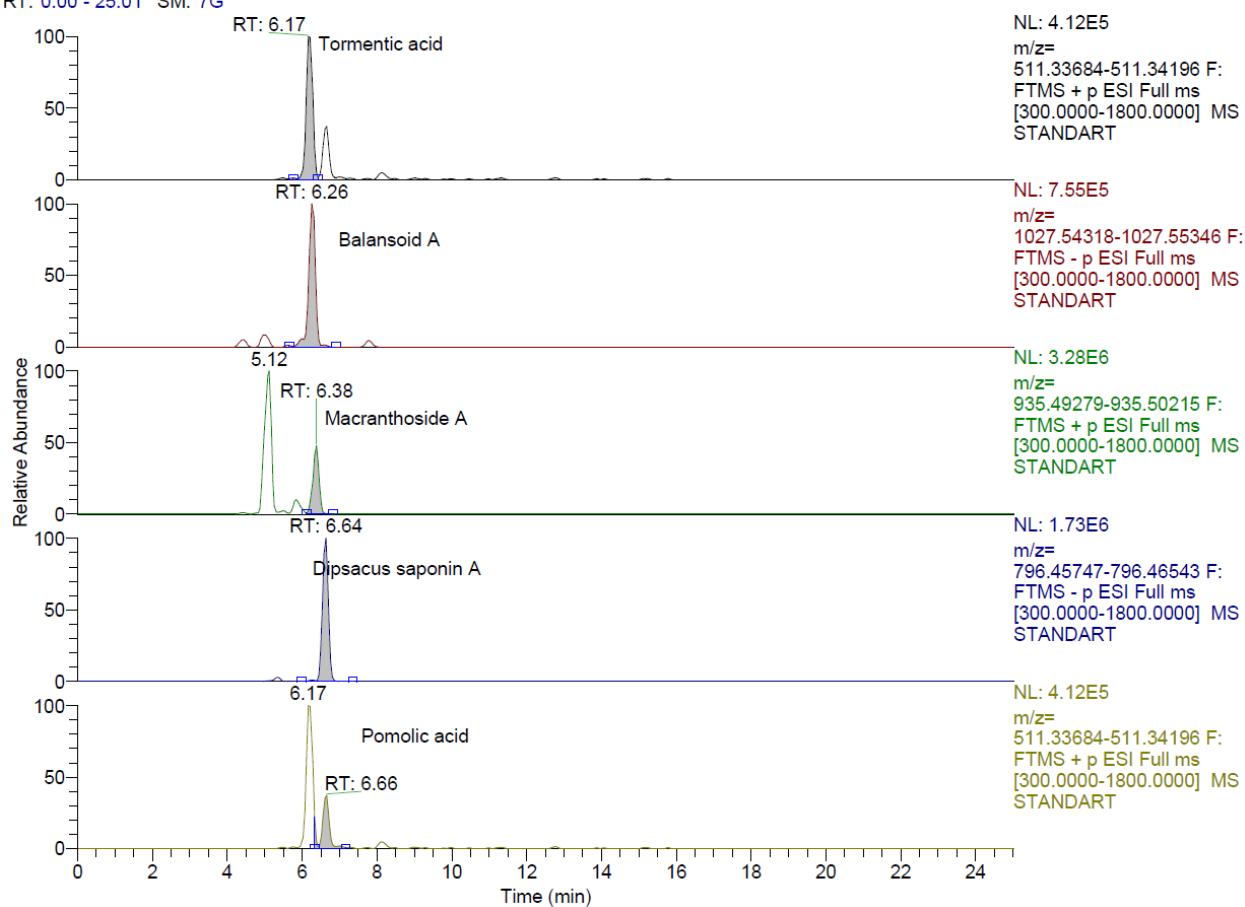


Figure S18: LC-HRMS chromatogram of standard mixture