Bioactive meroditerpenes from Cystoseira abies-marina, collected from the coast of S. Miguel island
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Introduction
The search of natural products with pharmacological properties has been increasing, particularly the discovery of molecules isolated from marine organisms [1]. Preliminary study on the extracts of the alga Cystoseira abies-marina, collected in S. Miguel Island, showed very promising results for antitumour and antioxidant activities [2]. These results have increased our interest in this alga and have encouraged their phytochemical study. Until now, five compounds were isolated and identified as benzoic acid (1),two new norsesquiterpenes [Cystoazores A (2) and Cystoazores B (3)], and two new meroditerpenes [Cystoazorone A (4) and Cytoazorone B (5)] from the extracts of this marine alga.

Results and discussion
The compounds isolated from Cystoseira abies-marina, Benzoic acid (1), Cystoazores A (2), Cystoazores B (3), Cystoazorone A (4) and Cytoazorone B (5) was tested for antioxidant, anticolinesterasic, antitumour and anti-inflammatory activities. The results are presented here.

Fig. 1. Antioxidant activity of compounds isolated from Cystoseira abies-marina presented in percentage. Trolox, Quercetin and BHT was standard solution. The maximum concentration used was 500 µg/mL.

Table 1. Inhibition growth and cytotoxicity activity of compounds isolated from Cystoseira abies-marina against HeLa and Vero cell line. Taxol was used as control.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Inhibition EC50 (µg/mL) (log base)</th>
<th>Cytotoxicity EC50 (µg/mL) (log base)</th>
<th>HeLa</th>
<th>Vero</th>
<th>HeLa</th>
<th>Vero</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoic acid</td>
<td>&gt;40</td>
<td>&gt;40</td>
<td>&gt;40</td>
<td>&gt;40</td>
<td>&gt;40</td>
<td>&gt;40</td>
</tr>
<tr>
<td>Cystoazores A</td>
<td>25±1,28</td>
<td>28±1,74</td>
<td>17,3±1,64</td>
<td>16,5±5,30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystoazores B</td>
<td>32,0±8,40</td>
<td>&gt;40</td>
<td>20,1±1,67</td>
<td>22,1±1,84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystoazorone A</td>
<td>10.2±0,19</td>
<td>16,7±0,08</td>
<td>2,8±1,16</td>
<td>6,9±0,52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoazorone B</td>
<td>&gt;40</td>
<td>&gt;40</td>
<td>&gt;40</td>
<td>&gt;40</td>
<td>&gt;40</td>
<td>&gt;40</td>
</tr>
<tr>
<td>Taxol</td>
<td>0.12±0,07</td>
<td>0,18±0,04</td>
<td>0,06±0,01</td>
<td>0,03±0,01</td>
<td></td>
<td></td>
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</tbody>
</table>

For antitumour activity, Cystoazorone A (4) was the most active compound in growth inhibition (cells in lag phase) and cytotoxicity (cells in log phase of growth) against HeLa tumour cells, followed by Cystoazores A (2). We can see that both compounds are selective for the tumour cell line (HeLa) and these compounds are more efficient in log phase of growth than in lag phase. This fact tells us, that the mode of action of active compounds 2 and 4 probably are related by cell division.

Fig. 2. Anticholinesterasic effect of the compounds isolated from Cystoseira abies-marina express in percentage. The α-pinene was used as control.

Cystoazorone A (4) and cytoazorone B (5) displayed the highest antioxidant activity, stronger than BHT (compound used in food industry). The results suggest that, at least as an industrial antioxidant (as opposed to antioxidants in vivo systems), the compounds appear to be useful (Fig. 1).

Fig. 3. Anti-inflammatory activity assay: inhibition of cyclooxygenases 1 and 2 (COX-1 and COX-2) by 100 µg/mL of compounds isolated from Cystoseira abies-marina. Results are expressed as percentage of inhibition.

Anti-inflammatory activity was tested by the inhibition of cyclooxygenases (COX1 and COX2) and lipoxgenase. As we can see in Fig. 3 benzoic acid (1), cystoazores A (4) and B (5) inhibited COX1 but cystoazores A (2) and B (3) demonstrated a stimulating effect. Benzoic acid (1), cystoazores B (3) and cystoazorone A (4) strongly inhibited COX2 and cystoazorone A (4) had a stimulating effect. Concerning lipoxgenase inhibition, cystoazorone A (4) was the only compound that presented some inhibitory activity, whilst the other compounds had a pro-inflammatory effect (Fig. 4).

Fig. 4. Anti-inflammatory activity: 5-lipoxygenase inhibition by compounds isolated from Cystoseira abies-marina. Results are expressed as percentage of inhibition. Quercetin was used as control.

Conclusions
Cystoazorone A and B are stronger antioxidants than BHT, therefore they have potential as natural antioxidants in food industry. Cystoazorone A was shown to be a good antitumour agent with good cytotoxicity and selectivity for tumor cells HeLa. Cystoazorone B and benzoic acid may be considered a good anti-inflammatory because, as we can see, they inhibit both cyclooxygenases (COX1 and COX2).

Material and Methods
Cystoseira abies-marina was collected in Mosteiroes, S. Miguel in Winter 2010 and Spring 2011. After grinding, Cystoseira abies-marina was exhaustively extracted with methanol and dichloromethane. The MeOH and CH2Cl2 extracts were evaporated to dryness, and then fractioned by column and preparative TLC chromatography on silica gel, eluting with solvent mixtures of different polarity. Protocol for antitumour activity, antioxidant activity and anticolinesterasic activity was made according Barreto et al. (2012)14 and Moujir et al. (2012)15. The anti-inflammation assays were carried out following the manufacturer’s protocols (Lipoxygenase Inhibitor Screening Assay Kit (LISA), Cayman Chemical Co., nº 760700) and (COX Inhibitor Screening Assay kit, Cayman Chemical Co., nº 560131).

Acknowledgements: Thanks are due University of Azores, FCT of Portugal (projects PTDC/MAR/100482/2008 and PEst-C/QUI/UNI0062/2011), and the Portuguese National MNR (FCT/2013), QOPNA, CIMIT and FEDER (Programa Polis) for financial support.

References