INTRODUCTION

Hypertension is a worldwide problem of epidemic proportions, affecting 15-20% of adults. It is the most common serious chronic health problem because it carries a high risk factor for arteriosclerosis, stroke, myocardial infarction and end-stage renal disease.

Angiotensin I - Converting Enzyme (ACE) belongs to the class of zinc metal proteases and is located in the vascular endothelial lining of the lungs (Miyoshi et al. 1991). This enzyme plays a key role in the control of blood pressure, by virtue of the rennin-angiotensin system (Ondetti et al. 1982; Rencland and Lithell 1994; Fujita and Yokoyama 2000). ACE acts as an exo-peptidase that cleaves a dipeptide of C-terminus of angiotensin I peptide to produce the potent vasoconstrictor angiotensin II (Curtiss et al. 1978; Maruyama et al. 1989; Dzau 2001) and inactivates bradykinin (Ukeda et al. 1991).

A number of ACE inhibitors have been extensively used in the treat of essential hypertension and heart failure in human; these include alacepril, benazepril, captopril, cilazapril, enalapril, fosinopril, lisinopril, moexipril, perindopril, quinapril, ramipril, tandolapril, and zofenopril (Kato and Suzuki 1971; Ondetti 1977; Sawayama et al. 1990). However, these commercial drugs are believed to cause certain side effects, such as cough, taste disturbances and skin rashes (Atkinson and Robertson 1979). Therefore, searching ACE inhibitors from natural resources have become more important.

Recently, many researchers have studied inhibitory activities on anti-ACE and antihypertensive effects after oral or intravenous administration in animal experiments and in clinical trials (Shin et al. 2001; Sato et al. 2002; Seppo et al. 2003). Also, much attention for the natural products and functional natural biomaterials has been paid to marine algae. In fact, for several centuries mankind has been exploiting the properties of certain foods to mitigate or prevent diseases (Joshipura et al. 2001). Marine algae have been consumed in Asian countries since ancient times, while in Western countries they have been utilized as sources of phycocollloids.

Screening of Extracts from Red Algae in Jeju for Potentials Marine Angiotensin - I Converting Enzyme (ACE) Inhibitory Activity

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This study was conducted to screen in vitro angiotensin - I converting enzyme (ACE) inhibitory activities of methanol (MeOH) and aqueous extracts at 20°C and 70°C, respectively, prepared from twenty-six red algae obtained from the coast of Jeju Island in Korea. Among aqueous extracts at 20°C (20AE) from red algae Lomentaria catenata showed the strongest ACE inhibitory activity and Lithophyllum okamurae recorded the second highest activity. From MeOH extract at 20°C (20ME) Ahnfeltiopsis flabelliformis possessed the strongest ACE inhibitory activity. Remarkable activities from MeOH extracts at 70°C (70ME) were observed in Grateloupia filicina, Sinkoraena lancifolia and Grateloupia lanceolata. However, no significant activity was found in aqueous extracts at 70°C (70AE). The IC₅₀ values, which are concentrations required to inhibit 50% activity of ACE, for ACE inhibitory activities of 20AE from Lithophyllum okamurae and L. catenata were 13.78 and 12.21 µg mL⁻¹, respectively. The IC₅₀ values of 20ME from A. flabelliformis and Laurencia okamurae were 13.84 and 106.15 µg mL⁻¹. Those of the 70ME from Bonnemaisonia hamifera, Grateloupia filicina, Sinkoraena lancifolia, G. lanceolata, Gracilaria verniculophylla and L. okamurae ranged from 25.82 to 124.69 µg mL⁻¹.

Key Words: ACE, Angiotensin I - Converting Enzyme, extract, Jeju, Red algae

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thickening, and gelling agent for various applications, including food industry. Dietary ingestion of seaweeds has been shown to decrease blood pressure in human (Seutsuna 1998) and fish cultivated (Mustafa and Nakagawa 1995; Mustafa et al. 1995). Nevertheless, the ACE inhibitory activity using algae has not been extensively studied.

The aim of the present study is to screen the ACE inhibitory activities of methanolic and aqueous extracts from indigenous red algae in Jeju Island.

**MATERIALS AND METHODS**

**Chemicals**

ACE (angiotensin I-converting enzyme) and HHL (hypueryl-histidyl-leucine) as a substrate of ACE were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were used reagent grade chemicals.

**Extract preparation**

Methanol extracts of the algae (1 g dry weight) were prepared with 100 mL of 80% methanol for 24 h under continuous shaking at a room (20°C) and high (70°C) temperature, and then aqueous extracts were prepared with the residue after MeOH extraction of the algae. The solvent of MeOH extract was volatilized in the fume hood (DAIHAN-LabTech®, Seoul, Korea) overnight, which led to the preparation of the aqueous extract.

**Red algal materials**

Red algae were collected from February to April, 2004 in the coast of Jeju Island, Korea. The algae were identified at the Faculty of Applied Marine Science in Cheju National University. Salt, epiphytes and sand were removed using freshwater. Finally the algae were carefully rinsed with freshwater and stored in a medical refrigerator at -20°C. The frozen samples were lyophilized and homogenized with a grinder before extraction.

**Table 1.** ACE inhibitory activities (%) of methanolic and aqueous extracts from red algae (tested concentration: 200 μg mL⁻¹).

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>20AE (a)</th>
<th>70AE (b)</th>
<th>20ME (c)</th>
<th>70ME (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porphyra tenera</td>
<td>24.59 ± 0.20</td>
<td>16.41 ± 0.30</td>
<td>20.79 ± 0.20</td>
<td>0.82 ± 0.80</td>
</tr>
<tr>
<td>Scinaia okamurae</td>
<td>17.02 ± 1.10</td>
<td>19.46 ± 0.10</td>
<td>42.84 ± 0.70</td>
<td>12.04 ± 0.30</td>
</tr>
<tr>
<td>Bonnemaisonia hamifera</td>
<td>8.05 ± 1.40</td>
<td>19.46 ± 0.20</td>
<td>43.41 ± 0.30</td>
<td>71.36 ± 0.80</td>
</tr>
<tr>
<td>Gelidium amansii</td>
<td>9.04 ± 1.70</td>
<td>12.99 ± 0.20</td>
<td>9.00 ± 0.50</td>
<td>17.93 ± 0.20</td>
</tr>
<tr>
<td>Pterocladiella capillacea</td>
<td>5.16 ± 1.60</td>
<td>17.17 ± 0.20</td>
<td>4.25 ± 0.10</td>
<td>13.37 ± 0.20</td>
</tr>
<tr>
<td>Lithophyllum okamurae</td>
<td>89.23 ± 1.00</td>
<td>8.05 ± 0.30</td>
<td>47.78 ± 0.10</td>
<td>30.29 ± 0.30</td>
</tr>
<tr>
<td>Carpopeltis affinis</td>
<td>3.30 ± 0.80</td>
<td>16.41 ± 0.20</td>
<td>38.09 ± 0.30</td>
<td>59.95 ± 0.50</td>
</tr>
<tr>
<td>Prioritis cornea</td>
<td>11.17 ± 1.40</td>
<td>1.59 ± 0.60</td>
<td>8.81 ± 0.30</td>
<td>25.54 ± 0.20</td>
</tr>
<tr>
<td>Grateloupiella filicina</td>
<td>7.48 ± 1.40</td>
<td>28.01 ± 0.30</td>
<td>17.93 ± 0.70</td>
<td>83.14 ± 1.00</td>
</tr>
<tr>
<td>Sinkorana lancefolia</td>
<td>3.68 ± 1.40</td>
<td>11.85 ± 0.30</td>
<td>43.60 ± 0.30</td>
<td>80.86 ± 1.00</td>
</tr>
<tr>
<td>Halymenia dilatata</td>
<td>23.83 ± 0.10</td>
<td>25.35 ± 0.10</td>
<td>23.45 ± 0.10</td>
<td>12.99 ± 0.30</td>
</tr>
<tr>
<td>Grateloupiella elliptica</td>
<td>10.33 ± 2.00</td>
<td>9.19 ± 0.30</td>
<td>9.76 ± 1.10</td>
<td>68.13 ± 1.10</td>
</tr>
<tr>
<td>Grateloupiella lanceolata</td>
<td>4.25 ± 1.60</td>
<td>19.84 ± 0.30</td>
<td>29.91 ± 0.10</td>
<td>89.04 ± 0.60</td>
</tr>
<tr>
<td>Gloiopeltis furcata</td>
<td>28.58 ± 1.60</td>
<td>23.26 ± 1.10</td>
<td>7.29 ± 0.30</td>
<td>31.05 ± 0.20</td>
</tr>
<tr>
<td>Schizymena dubyi</td>
<td>4.21 ± 1.50</td>
<td>7.29 ± 0.30</td>
<td>3.87 ± 0.40</td>
<td>26.11 ± 0.10</td>
</tr>
<tr>
<td>Placelocarpus sp.</td>
<td>22.50 ± 0.10</td>
<td>15.46 ± 0.20</td>
<td>14.70 ± 0.30</td>
<td>6.72 ± 1.00</td>
</tr>
<tr>
<td>Graclariarla texturii</td>
<td>65.40 ± 1.70</td>
<td>18.13 ± 2.60</td>
<td>19.27 ± 0.70</td>
<td>41.32 ± 0.30</td>
</tr>
<tr>
<td>Gracilariarla verrucosa</td>
<td>13.56 ± 1.50</td>
<td>22.69 ± 0.10</td>
<td>7.29 ± 1.30</td>
<td>74.02 ± 1.10</td>
</tr>
<tr>
<td>Alnfteltiparis fflabiliform is</td>
<td>73.45 ± 0.80</td>
<td>6.91 ± 0.30</td>
<td>97.59 ± 1.10</td>
<td>17.74 ± 0.10</td>
</tr>
<tr>
<td>Chordrus crisps</td>
<td>12.42 ± 1.40</td>
<td>15.27 ± 0.20</td>
<td>26.30 ± 0.10</td>
<td>27.63 ± 0.20</td>
</tr>
<tr>
<td>Lomentariar catenata</td>
<td>98.92 ± 1.10</td>
<td>24.59 ± 0.10</td>
<td>12.23 ± 0.20</td>
<td>6.15 ± 0.70</td>
</tr>
<tr>
<td>Martensiaria denticulata</td>
<td>25.35 ± 0.10</td>
<td>13.18 ± 0.20</td>
<td>16.03 ± 0.20</td>
<td>24.78 ± 0.10</td>
</tr>
<tr>
<td>Chordria cassiclas</td>
<td>32.19 ± 0.50</td>
<td>18.13 ± 0.20</td>
<td>58.43 ± 0.50</td>
<td>42.46 ± 0.30</td>
</tr>
<tr>
<td>Laurencia okamurae</td>
<td>15.08 ± 0.90</td>
<td>28.20 ± 0.10</td>
<td>78.01 ± 0.80</td>
<td>69.08 ± 2.10</td>
</tr>
<tr>
<td>Chondrophyccus undulatus</td>
<td>16.79 ± 0.20</td>
<td>2.73 ± 0.50</td>
<td>12.42 ± 0.30</td>
<td>3.68 ± 1.10</td>
</tr>
<tr>
<td>Polysiphonia japonica</td>
<td>-</td>
<td>-</td>
<td>19.46 ± 0.20</td>
<td>6.15 ± 0.60</td>
</tr>
</tbody>
</table>

(a) 20AE: aqueous extract at 20°C, (b) 70 AE: aqueous extract at 70°C, (c) 20ME: methanolic extract at 20°C, (d) 70 ME: methanolic extract at 70°C, (e) Mean ± SE of determinations was made in triplicate experiments.
after being dissolved in distilled water. The aqueous extracts were concentrated under a vacuum in a rotary evaporator at 40°C to adjust to a concentration of 200 µg mL\(^{-1}\).

**ACE inhibitory activity assay**

The ACE inhibitory activity assay was carried out using modified methods of Cushman and Cheung (1971) with some modifications. A two hundred microliter of the substrate (5 mM Hip-His-Leu in 100 mM sodium borate buffer containing 300 mM NaCl at pH 8.3) with 80 µL of the sample solution was pre-incubated at 37°C for 3 min, and the mixture was incubated with 20 µL ACE enzyme (100 mU mL\(^{-1}\)) for 30 min at the same temperature. The reaction was terminated by adding 250 µL of 1 M HCl. The released hippuric acid from the substrate was extracted with 1.7 mL of ethyl acetate (EtOAc). After centrifugation (800 \(\times\) g, 15 min), 1 mL of the upper layer was transferred into a tube and evaporated at the room temperature for approximately 40 min using a vacuum evaporator. The hippuric acid was dissolved in 1 mL of double distilled water and the absorbance was read at 228 nm using an UV-spectrophotometer.

The reaction was expressed as percentage inhibition and calculated from the equation:

\[
\text{Inhibition} \% = \left(1 - \frac{S_A - S_B}{C}\right) \times 100
\]

Where \(S_A\) = absorbance of sample, \(S_B\) = absorbance of blank (no ACE) and \(C\) = absorbance of control reaction (no sample or ACE inhibitor). And IC\(_{50}\) values, which are concentrations required to inhibit 50% activity of ACE, were obtained from the samples that possessed remarkable ACE inhibitory activity.

**RESULTS AND DISCUSSION**

A total of 104 samples from 26 marine red algae, including 20AE (aqueous extract at 20°C), 20ME (methanolic extract at 20°C), 70AE (aqueous extract at 70°C), 70ME (methanolic extract at 70°C) have been screened for their potential ACE inhibitory activities. The results are summarized in Table 1. The 20AE from *Lomentaria catenata* exhibited the strongest ACE inhibitory activity (98.92% ± 1.1, mean ± SE). At the 20AE *Lithophyllum okamurae* possessed the second highest ACE inhibitory activity (89.23% ± 1.0). Also the 20AE from *Ahnfeltiopsis flabelliformis* and *Gracilaria textorii* showed...
moderate activities (73.45% ± 0.8 and 65.40% ± 1.7, respectively). However, the other 20AE were showed poor activities with 50% or less. In the 70AE no ACE inhibitory activity was observed (less than 30%).

On the other hand, the 20ME from *Ahnfeltiopsis flabelliformis* was the strongest in the activity (97.59% ± 1.1). The other extracts were less than 60% except *Laurencia okamurae* (78.01% ± 0.8). From the 70ME from *Grateloupia filicina*, *Sinkoraena lancifolia* and *Grateloupia lanceolata* indicated significant ACE inhibitory activities (83.14% ± 1.0, 80.86% ± 1.0 and 89.04% ± 0.6, respectively). The other 70ME from *Bonnemaisonia hamifera*, *Gracilaria verrucosa* and *Laurencia okamurae* were also showed moderate ACE inhibitory activities (71.36% ± 0.8, 74.02% ± 1.1 and 69.08% ± 2.1, respectively).

Many studies have been recently reported for ACE inhibitors using the extract of marine animals. Marine clam protein hydrolysates prepared with thermolysin had 0.748 µg mL−1 of IC50 value in ACE inhibitory activity (Lee et al. 2002). Kim et al. (2002) prepared krill hydrolysates with Alcalase and isolated the anti-ACE active fraction with 500 Da of molecular weight that possessed 0.57 mg mL−1 of IC50 value. And the yellowfin sole frame protein with a molecular mass of 1.3 kDa consisting of 11 amino acids had a strong ACE inhibitory activity and its IC50 value was 28.7 µg mL−1 (Jung et al. 2004). The purified peptide from fermented oyster sauce had an IC50 value of 87.40 µg mL−1 (Je et al. 2005). The above two researches evaluated antihypertensive effect in spontaneous hypertensive rats (SHRs) following oral administration with anti-ACE peptide or hydrolysates from marine animals and observed a significant reduc-

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**Fig. 3.** ACE inhibitory activities for 70ME from (A) *Bonnemaisonia hamifera*, (B) *Grateloupia filicina*, (C) *Sinkoraena lancifolia*, (D) *Grateloupia lanceolata*, (E) *Gracilaria verrucosa* and (F) *Laurencia okamurae*, and their IC50 values.
tion in rat blood pressure. However, only a few studies regarding ACE inhibition from marine algae were characterized for the investigation of anti-ACE activities. For examples, Ecklonia cava exhibited an IC\textsubscript{50} of around 0.3 µg mL\textsuperscript{-1} for anti-ACE (Athukorala and Jeon 2005), and the peptides separated from Undaria pinnatifida significantly decreased blood pressure in SHRs after oral administration (Nakano et al. 1998; Kunio and Takahisa 2000; Sato et al. 2002; Kunio et al. 2004).

As shown in the Fig. 1, the IC\textsubscript{50} value of ACE inhibitory activities from marine algae tested in the present study ranged from 12.21-124.69 µg mL\textsuperscript{-1} (Fig. 1-3). The 20AE of Lithophyllum okamurae and L. catenata recorded around 13 µg mL\textsuperscript{-1} and the 20ME of A. flabelliformis and Laurencia okamurae from 13.84 to 106.15 µg mL\textsuperscript{-1} (Fig. 2). And the 70ME from six the marine red algae from 25.82 to 124.69 µg mL\textsuperscript{-1} (Fig. 3) as an IC\textsubscript{50} value for ACE inhibitory activity. The strongest ACE inhibitory activity was found in aqueous extract at 20°C (20AE) from L. catenata (12.21 µg mL\textsuperscript{-1}).

According to Athukorala and Jeon (2005), commercial ACE inhibitor, captopril had an IC\textsubscript{50} value of 0.05 ± 0.8 µg mL\textsuperscript{-1}, even though the samples tested in this study were just crude extracts under unpurified state it had high activity. The anti-ACE activities of the extracts from some marine red algae were remarkable, compared to the purified and fractionated peptides from marine organisms (Kunio and Takahisa, 2000). This suggests that there are excellent potential ACE like-inhibitors derived from marine red algae. In our previous study, green and brown algae indicated good anti-ACE activity, especially in 70ME of some brown algae (Cha et al. 2006). We concluded marine algae might have good ACE like-inhibitors that are associated with not only proteins but also fucoxanthin (K. Ikeda et al. 2003), phlorotannin (J.C Liu et al. 2003) and polyphenolic compounds (Ángeles M. et al. 2007).

In the present results remarkable anti-ACE activities were observed in both 20AE and 70ME from a few species as mentioned the above. This implies that red algae also have ACE-like-inhibitors. Therefore, further studies looking for ACE inhibitory active compounds from marine algae are required.

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