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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 IC_{50} 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 IC_{50} 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 vermiculophylla* and *L. okamurae* ranged from 25.82 to 124.69 μg mL^{-1}.

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

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.

A number of investigators have studied the various bioactivities of algae and have found that seaweeds have not only nutritional effects but also beneficial properties to cure various diseases and keep good health (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 phycocolloids,

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Table 1. ACE inhibitory activities (%) of methanolic and aqueous extracts from red algae (tested concentration: 200 μg mL^{-1}).

Scientific name	ACE inhibition activity (%)			
	20AE[a)]	70AE[b)]	20ME[c)]	70ME[d)]
Porphyra tenera	24.59 ± 0.20[e)]	16.41 ± 0.30	20.79 ± 0.20	0.82 ± 0.80
Scinaia okamurae	17.02 ± 1.10	19.46 ± 0.10	42.84 ± 0.70	12.04 ± 0.30
Bonnemaisonia hamifera	8.05 ± 1.40	19.46 ± 0.20	43.41 ± 0.30	71.36 ± 0.80
Gelidium amansii	9.04 ± 1.70	12.99 ± 0.20	9.00 ± 0.50	17.93 ± 0.20
Pterocladiella capillacea	5.16 ± 1.60	17.17 ± 0.20	4.25 ± 0.10	13.37 ± 0.20
Lithophyllum okamurae	89.23 ± 1.00	8.05 ± 0.30	47.78 ± 0.10	30.29 ± 0.30
Carpopeltis affinis	3.30 ± 0.80	16.41 ± 0.20	38.09 ± 0.30	59.95 ± 0.50
Prionitis cornea	11.17 ± 1.40	1.59 ± 0.60	8.81 ± 0.30	25.54 ± 0.20
Grateloupia filicina	7.48 ± 1.40	28.01 ± 0.30	17.93 ± 0.70	83.14 ± 1.00
Sinkoraena lancifolia	3.68 ± 1.40	11.85 ± 0.30	43.60 ± 0.30	80.86 ± 1.00
Halymenia dilatata	23.83 ± 0.10	25.35 ± 0.10	23.45 ± 0.10	12.99 ± 0.30
Grateloupia elliptica	10.33 ± 2.00	9.19 ± 0.30	9.76 ± 1.10	68.13 ± 1.10
Grateloupia lanceolata	4.25 ± 1.60	19.84 ± 0.30	29.91 ± 0.10	89.04 ± 0.60
Gloiopeltis furcata	28.58 ± 1.60	23.26 ± 1.10	7.29 ± 0.30	31.05 ± 0.20
Schizymenia dubyi	4.21 ± 1.50	7.29 ± 0.30	3.87 ± 0.40	26.11 ± 0.10
Phacelocarpus sp.	22.50 ± 0.10	15.46 ± 0.20	14.70 ± 0.30	6.72 ± 1.00
Gracilaria textorii	65.40 ± 1.70	18.13 ± 2.60	19.27 ± 0.70	41.32 ± 0.30
Gracilaria verrucosa	13.56 ± 1.50	22.69 ± 0.10	7.29 ± 1.30	74.02 ± 1.10
Ahnfeltiopsis flabelliformis	73.45 ± 0.80	6.91 ± 0.30	97.59 ± 1.10	17.74 ± 0.10
Chondrus crispus	12.42 ± 1.40	15.27 ± 0.20	26.30 ± 0.10	27.63 ± 0.20
Lomentaria catenata	98.92 ± 1.10	24.59 ± 0.10	12.23 ± 0.20	6.15 ± 0.70
Martensia denticulata	25.35 ± 0.10	13.18 ± 0.20	16.03 ± 0.20	24.78 ± 0.10
Chondria cassicaulis	32.19 ± 0.50	18.13 ± 0.20	58.43 ± 0.50	42.46 ± 0.30
Laurencia okamurae	15.08 ± 0.90	28.20 ± 0.10	78.01 ± 0.80	69.08 ± 2.10
Chondrophycus undulatus	16.79 ± 0.20	2.73 ± 0.50	12.42 ± 0.30	3.68 ± 1.10
Polysiphonia japonica	-	-	19.46 ± 0.20	6.15 ± 0.60

[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.

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 (hyppuryl-histidyl-leucine) as a substrate of ACE were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were used reagent grade chemicals.

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.

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 a 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,

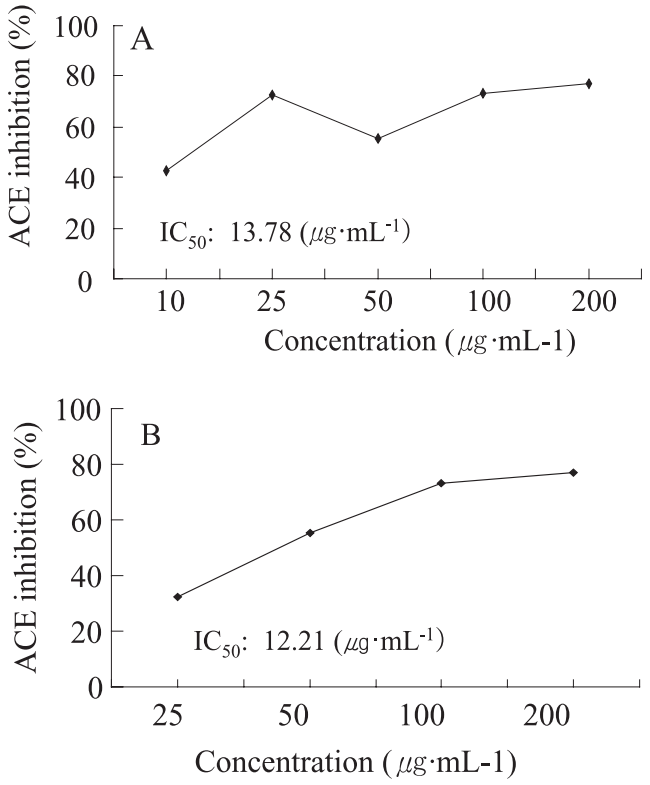

Fig. 1. ACE inhibitory activities for 20AE from (A) *Lithophyllum okamurae* and (B) *Lomentaria catenata*, and their IC_{50} values.

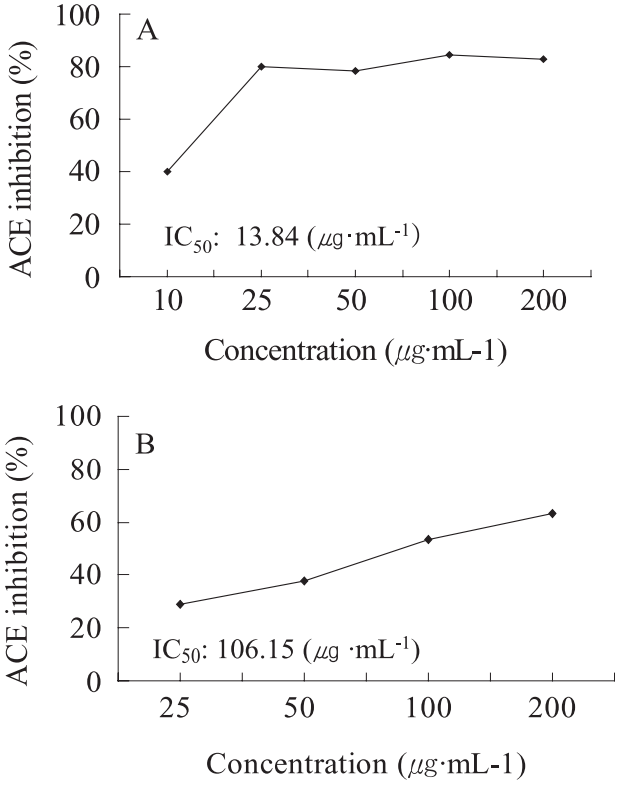

Fig. 2. ACE inhibitory activities for 20ME from (A) *Ahnfeltiopsis flabelliformis* and (B) *Laurencia okamurae*, and their IC_{50} values.

after then 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 × 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:

$$\text{Inhibition (\%)} = \{1 - (S_A - S_B)/C\} \times 100$$

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

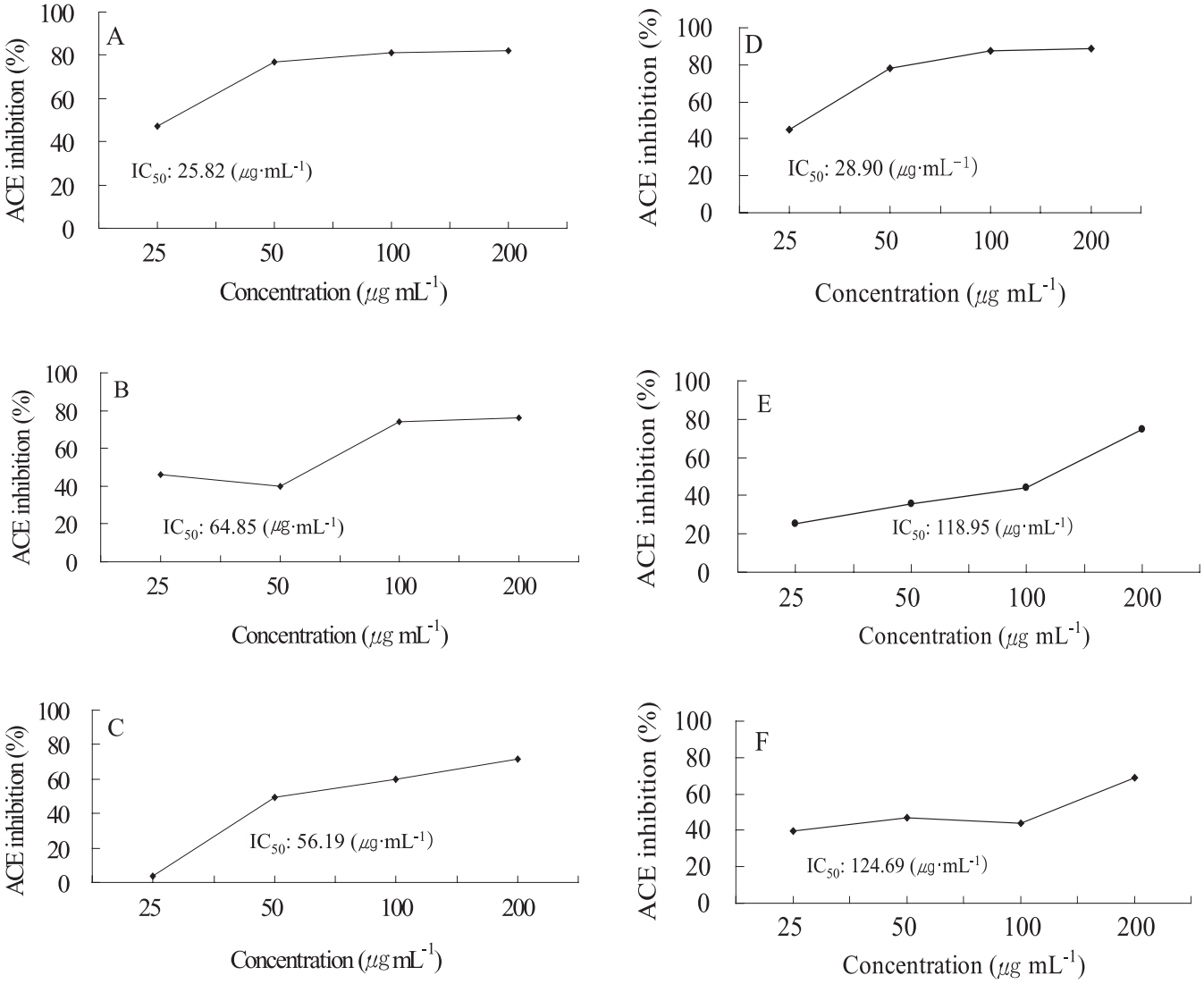

Fig. 3. ACE inhibitory activities for 70ME from (A) *Bonnemaisonia hamifera*, (B) *Grateloupia filicina*, (C) *Sinkoraena lancifolia*, (D) *Grateloupia lanceolata*, (E) *Gracilaria vermiculophylla* and (F) *Laurencia okamurae*, and their IC_{50} values.

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 IC_{50} 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 IC_{50} 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 IC_{50} value was 28.7 μg mL^{-1} (Jung *et al*. 2004). The purified peptide from fermented oyster sauce had an IC_{50} 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-

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_{50} of around 0.3 μg mL^{-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_{50} value of ACE inhibitory activities from marine algae tested in the present study ranged from 12.21-124.69 μg mL^{-1} (Fig. 1-3). The 20AE of *Lithophyllum okamurae* and *L. catenata* recorded around 13 μg mL^{-1} and the 20ME of *A. flabelliformis* and *Laurencia okamurae* from 13.84 to 106.15 μg mL^{-1} (Fig. 2). And the 70ME from six the marine red algae from 25.82 to 124.69 μg mL^{-1} (Fig. 3) as an IC_{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^{-1}).

According to Athukorala and Jeon (2005), commercial ACE inhibitor, captopril had an IC_{50} value of 0.05 ± 0.8 μg mL^{-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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REFERENCES

Ángeles M., Bayón P., Alcaíde J.M., Polo M.C. and Pueyo E. 2007. Angiotensin I - converting enzyme inhibitory compounds in white and red wines. *Food Chem*. **100:** 43-47

Athukorala Y. and Jeon Y.-J. 2005. Screening for Angiotensin 1-Converting Enzyme Inhibitory Activity of *Ecklonia cava*. *J. Food Sci. and Nutr*. **10:** 134-139.

Atkinson A.B. and Rovertson J. 1979. Captopril in the treatment of clinical hypertension and cardiac failure. *Lanc*. **2:** 836-839.

Cha S.H., Ahn G.N., Heo S.J., Kim K.N., Lee K.W., Song C.B., Cho S.K. and Jeon Y.J. 2006. Screening of extracts from Marine Green and Brown Algae in Jeju for Potential Marine Angiotensin - I Converting Enzyme (ACE) Inhibitory Activity. *J. Korean Soc. Food Sci. Nutr*. **35:** 307-314.

Curtiss C., Chon J.N., Vrobel T. and Francious J.A. 1978. Role of the rennin-angiotensin system in the systemic vasoconstriction of chronic congestive heart failure. *Circulation* **58:** 763-770.

Cushman D.W. and Cheung H.S. 1971. Spectrophotometric assay and properties of the angiotensin-conveting enzyme of rabbit lung. *Biochemical Pharmacology* **20:** 1637-1648.

Dzau V.J. 2001. Tissue Angiotensin and Pathobiology of Vascular Disease: A Unifying Hypothesis. *Hypertension* **37:** 1047-1052.

Fujita H and Yokoyama M. 2000. Classification and antihypertensive activity of angiotensin I - converting enzyme inhibitory peptide derived from food proteins. *J. Food Sci*. **65:** 564-569.

Ikeda K., Kitamura A., Machida H., Watanabe M., Negishi H., Hiraoka J. and Nakano T. 2003. Effect of *Undaria pinnatifida* (WAKAME) on the development of cerebrovascular disease in stroke-prone spontaneously hypertensive rats. *Clin. Exp. Pharm. and physic*. **30:** 44-48

Je J.Y., Park P.J., Byun H.G., Jung W.K. and Kim S.K. 2005. Angiotensin I converting enzyme (ACE) inhibitory peptide derived from the sauce of fermented blue mussel, Mytilus edulis. *Bior. Tech*. **96:** 1624-1629.

Joshipura K.J., Hu F.B. and Manson J.E. 2001. The effect of fruit and vegetable intake on risk for coronary heart disease. *Ann. Intern. Med*. **134:** 1106-1114.

Jung W.K., Je J.Y., Park P.J., Son B.W., Kim H.C., Choi Y.K. and Kim S.K. 2004. Angiotensin I-converting enzyme inhibitory peptide from yellowfin sole (*Limanda aspe*ra) frame protein and its antihypertensive effect in spontaneously hypertensive rats. *Food Che*. **94:** 26-32.

Kato H. and Susuki T. 1971. Bradykinin-potentiatinf peptides from venom of *Agkistrodon- halys blomhoffii*: Isolation of five bradykinin potentiators B and C. *Bioche*. **10:** 972-980.

Kim D.-S., Park D.-C. and Do J.-R. 2002. Angiotensin I converting enzyme inhibitory activity of Krill (*Euphausia superba*) Hydrolysate. *Fisheries Sci. and tech*. **5:** 21-27.

Kunio S. and Takahisa N. 2000. Identification of an antihypertensive peptide from peptic digest of Wakame (*Undaria pin-*

natifida). *J. Nutr. Biochem*. **11:** 450-456.

Kunio S., Keisei M. and Chen J.R. 2004. Antihypertensive effects of *Undaria pinnatifida* (Wakame) peptide on blood pressure in spontaneously hypertensive rats. *J. Nutr. Biochem*. **15:** 267-272.

Lee T.G, Yeum D.M. and Kim S.B. 2002. Characteristics of angiotensin converting enzyme inhibitory peptides from thermolysin hydrolysate of manila clam, Ruditapes philippinarum proteins. *J. of Korean Fish. Soc*. **35:** 529-533.

Liu J.C., Hsu F.L., Tsai J.C., Chan P., Liu J.Y., Thomas G.N., Tomlinson B., Lo M.Y. and Lin J.Y. 2003. Antihypertensive effects of tannins isolated from traditional Chinese herbs as non-specific inhibitors of angiontensin converting enzyme. *Life Sci.* **73:** 1543-1555.

Maruyama S., Mitachi H., Awaya J., Kurono M., Tomizuka N. and Suzuki H. 1989. Angiotensin I - converting enzyme inhibitory activity of the C-terminal hexapeptide of αs1-casein. *Agri. and Biol. Chem.* **53:** 2107-2114.

Miyoshi S., Ishikawa H., Kaneko T., Fukui F., Tanaka H. and Maruyama S. 1991. Structure and activity of angiotensin-converting enzyme inhibitors in an α-zein hydrolysate. *Agr. and Biol. Chem.* **55:** 1313-1318.

Mustafa M.G. and Nakagawa H. 1995. A review: Dietary benefits of algae as an additive in fish feed. *The Isreali J. of Aqua*. **47:** 155-162.

Mustafa M.G., Wakamatsu S., Takeda T.A., Umino T. and Nakagawa H. 1995. Effects of algae meal as feed additive on growth, feed efficiency, and body composition in Red Sea Bream. *Fish. Sci.* **61:** 25-28.

Nakano T., Hidaka H., Ucjida J., Nakajima K. and Hata Y. 1998. Hypetensive effects of wakame. *J. Jpn. Soc. Clin. Nutr*. **20:** 92.

Ondetti M.A. 1977. Desine of specific inhibitors of angiotensin-coverting enzyme: New ckass of orally active antihypetensive agents. *Sci.* **196:** 441-444.

Ondetti M.A., Rubin B. and Cushman D.W. 1982. Enzyme of the rennin-angiotensin system and their inhibitors. *Annu. Rev. Biochem*. **51:** 283-308.

Rencland R. and Lithell H. 1994. Angiotensin-converting enzyme in human skeletal muscle. A simple *in vitro* assay of activity in needle biopsy specimens. *Scand. J. Clin. Lab. Invest.* **54:** 105-111.

Sato M., Hoskawa T., Yamagichi T., Nakano T., Muramoto K. and Kahara T. 2002. Angiotensin I-converting enzyme inhibitory peptides derived from Wakame (*Undaria pinnatifida*) and their antihypertensive effect in spontaneously hypertensive rats. *J of Agri. and Food Chem*. **50:** 6245-6252.

Sawayama T, Itokawa A., Shumada K., Doi Y., Kimura K. and Nishimura H. 1990. Synthesis of 1-[(s)-acetylthio-2-methyl-propanoyl]-L-propyl-L-phenylanine (Alacepril) and one of its active metabolites, the desacetyl derivation (DU-1227). *Chem. Pharm. Bull*. **38:** 529-531.

Seppo L., Jauhiainen T., Poussa T. and Korpela R. 2003. A fermented milk high in bioactive peptides has a blood pressure-lowering effect in hypertensive subjects. *American J. of Clin. Nutr*. **77:** 326-330.

Shin Z.-I., Yu R., Park S.-A., Chung D.-K., Nam S.-H. and Kim K.-S. 2001. His-His-Leu, an angiotensin I-converting enzyme inhibitory peptide derived from Korean soybean paste, exerts antihypertensive activity *in vivo*. *J. of Agr. and Food Chem*. **49:** 3004-3009.

Ukeda H., Matsuda H., Kuroda H., Osajima K., Matsufuji H. and Osajima Y. 1991. Preparation and separation of angiotensin I converting enzyme inhibitory peptides. *Nippon Noge. Kai*. **65:** 1223-1228.

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