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### Pharmacological review on Natural ACE inhibitors

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#### Abstract

Hypertension is one of the most common worldwide diseases that afflict humans. Angiotensin-I converting enzyme (ACE) plays an important role in the regulation of blood pressure and hypertension. ACE catalyzes the conversion of inactive angiotensin-I into a potent vasoconstrictor, angiotensin-II. ACE also inactivates the vasodilator, bradykinin. Inhibitors of ACE are often used to treat myocardial infarction, hypertension, and other cardio-related diseases. Angiotensin II form by angiotensin I catalyzes by angiotensin-converting enzyme (ACE) cause vasoconstriction, and inactivation of vasodilator, bradykinin. Angiotensin II results in increases peripheral resistance, heart rate, and cardiac output. The influences of ACE on blood pressure make it an ideal target clinically and nutritionally in the treatment of hypertension. Inhibition of ACE mainly results in an overall antihypertensive effect. Several synthetic ACE inhibitors such as captopril, enalapril, lisinopril and temocapril are in clinical use for the treatment of hypertension. Synthetic ACE inhibitors are believed to have certain side effects such as cough, taste disturbances, skin rashes and high cost and drug-drug interactions. Therefore, search for non-toxic, safer, innovative and economical ACE inhibitors as alternatives to synthetic drugs. It is of great interest among researchers and many natural ACE inhibitors have been isolated from functional food and natural bio-resources. The natural ACE inhibitors are considered to be milder and safer compared with synthetic drugs. This review focuses on phytochemicals, chemistry, mechanism, and lead compounds derived from plants and use to control or in the treatment of hypertension. Detailed accounts from various natural products are study below.

**Keywords:** Angiotensin converting enzyme, bioactive peptides, Flavonoids, Vasoconstrictor, Aldosterone.

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## INTRODUCTION

### **Renin-angiotensin system (RAS)**

The renin-angiotensin system plays an important role in an interrelated set of mechanism for the control of the volume, pressure, and electrolyte composition of blood, [1, 2] and may play a role in the pathogenesis of aspects of the metabolic syndrome. [3]

### **Angiotensin converting enzyme-**

ACE (also known as ACE, kininase II [4, 5] or dipeptidyl carboxypeptidase) also a zinc-containing enzyme - which hydrolyses a carboxyl terminal peptide.

Angiotensin I is relatively inactive and is activated by being turned into angiotensin II by angiotensin-converting enzyme. Angiotensin II cause vasoconstriction [6, 7] (constriction of the blood vessels) and stimulation of the synthesis of aldosterone by the adrenal cortex. [1].

Angiotensin I-converting enzyme (ACE; EC 3.4.15.1) plays an important physiological role in the regulation of blood pressure and electrolyte homeostasis [8].

### **Inhibitors of Angiotensin converting enzyme-**

In the 1970s, Ferreira in collaboration with Greene and, finally by Erdos and others investigators, two classes of inhibitors of the renin-angiotensin system were identified: angiotensin II antagonists and converting enzyme inhibitors, which slow the rate of formation of angiotensin II from its inactive precursor. An important competitive inhibitor of ACE is captopril, which inhibits conversion of the relatively inactive angiotensin I to the angiotensin II [1].

Angiotensin-converting enzyme (ACE) inhibitors act as vasodilators [4], but the most obvious potential benefit is their effect on the renin-angiotensin-aldosterone system by reducing the levels of Ang. II. Clinical studies have demonstrated that ACE inhibitors significantly reduce the incidence of patients with myocardial infarction, ischemic events in patients with coronary artery disease. [7]

Clinical studies have demonstrated that ACE inhibitors significantly reduce the morbidity and mortality of patients with myocardial infarction or heart failure. (9). ACE inhibitors are esterified pro-drugs which undergo enzymatic *in vivo* hydrolysis to be converted into pharmacologically active metabolites, i.e., the corresponding di-acid forms (10).

### **Examples of ACE inhibitors-[11]**

ACE inhibitors can be divided into three groups based on their molecular structure.

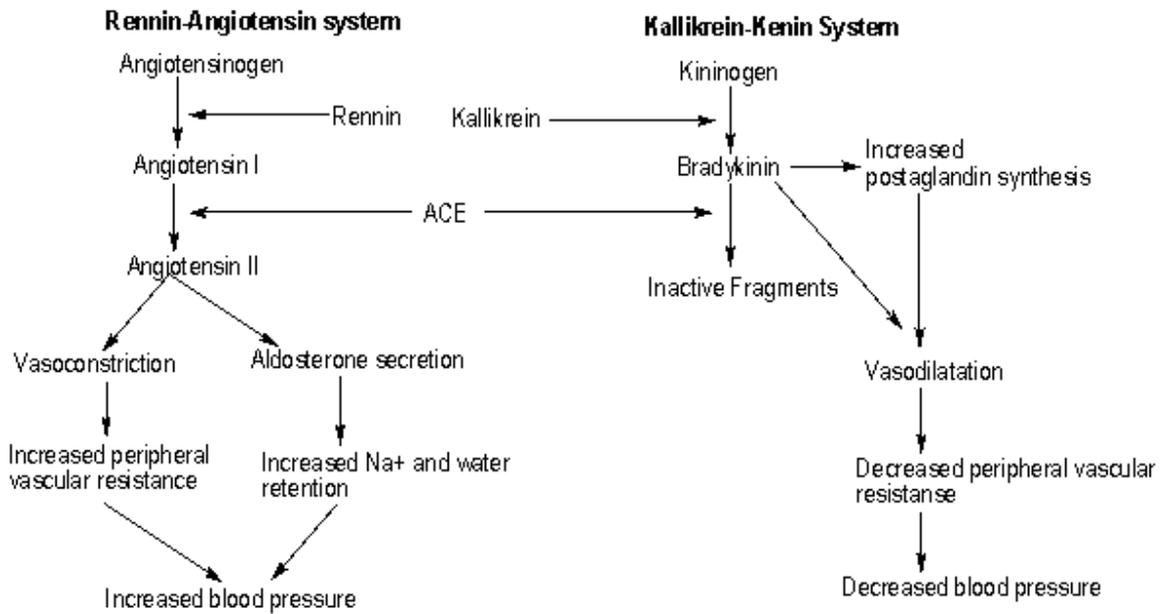
**Sulfhydryl-containing agents;** Captopril (trade name Capoten), the first ACE inhibitor, Zofenopril

**Dicarboxylate-containing agents;** this is the largest group, including: Enalapril, Ramipril, Quinapril, Perindopril, Lisinopril, Benazepril

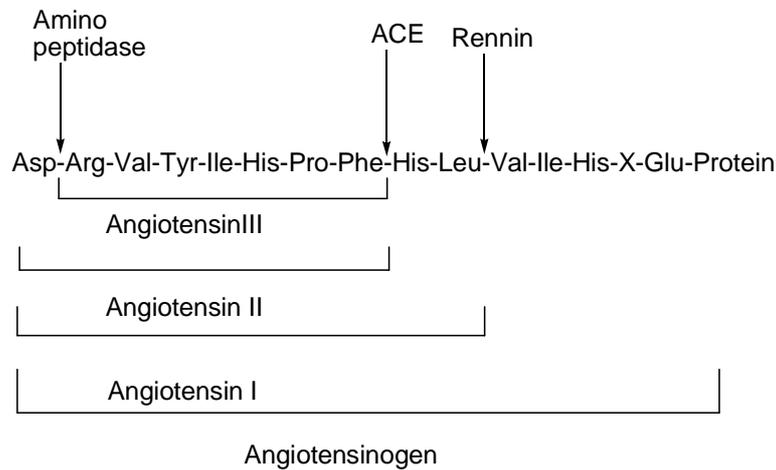
**Phosphonate-containing agents;** Fosinopril

**Uses for ACE inhibitors**

ACE inhibitors to prevent, treat or improve symptoms in conditions such as: High blood pressure, coronary artery disease, heart failure, diabetes, certain chronic kidney diseases, heart attacks, scleroderma, migraines. ACE inhibitors are usually taken once daily, and many people take them in the morning. [3, 6, 11, 13, 12, 14]



**Fig. 1. Role of angiotensin converting enzyme(ACE) in blood pressure regulation**



**Fig.2. Sites of stepwise enzymatic cleavage of human angiotensionogen**

**Drug Interactions for ACE inhibitors**

<b>Drug</b>	<b>ACE inhibitors</b>	<b>Result of interaction</b>
Allopurinol	Captopril	Increased risk of hypertension
Antacids	All	Decreased bioavailability of ace inhibitors (more likely with captopril & fosinopril)
Capsaicin	All	Exacerbation of cough
Digoxin	All	Either increased or decreased plasma digoxin levels
Diuretics	All	Potential excessive reduction in B.P. ;the effects of loop diuretics may be reduced
Iron salts	Captopril	Reduction of captopril levels unless administration is separated by at least 2 hours
Potassium-sparing diuretics	All	Elevated serum potassium levels
Lithium	All	Increased serum lithium levels
NSAIDs	All	Decreased hypotensive effects
Phenothiazides	All	Increased pharmacological effects ace inhibitors
Probenecid	Captopril	Decreased clearance & increased blood levels of captopril
Rifampin	Enalapril	Decreased pharmacological effects of enalapril
Tetracycline	Quinapril	Decreased absorption of tetracycline(may result from high magnesium content of quinapril tablets

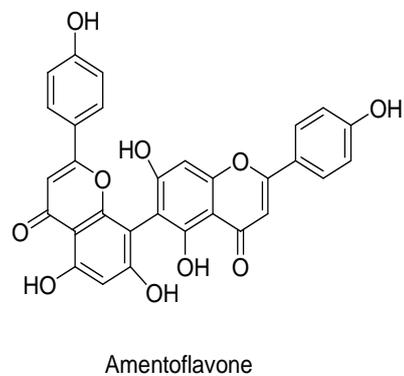
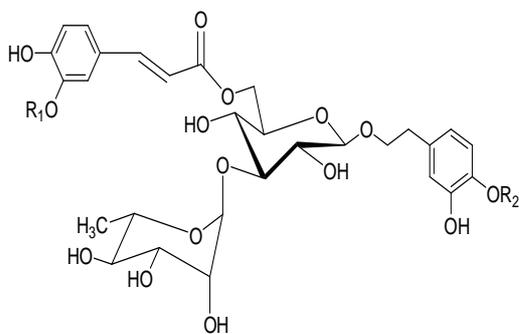
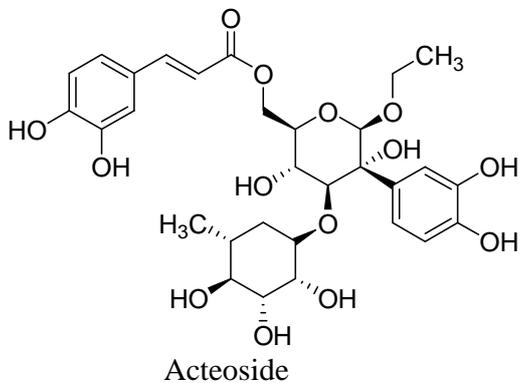
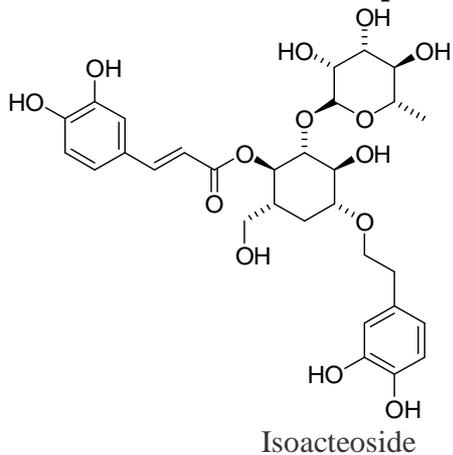
**Side effects and cautions**

Commonly prescribe ACE inhibitors because they don't often cause side effects. The most common side effect is a dry cough. Possible, although rare, side effects include: increased blood-potassium level (hyperkalemia), rash, dizziness, lightheadedness, changes in taste, reduced appetite over long intervals. [11, 15]

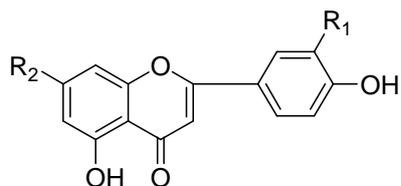
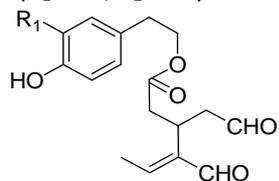
**Clinically significant drug interactions of ACE inhibitors**

Signs and symptoms of overdose of ACE inhibitor can be similar to the medication side effects, but are usually more severe. A physician should be called immediately if the patient shows any of the following: Low pressure blood (hypotension), Edema (swelling) in the face, mouth, throat, hands or feet, Fainting (syncope), Fever or chills, Sore throat, Convulsions or seizures, Coma. [15, 16, 17]

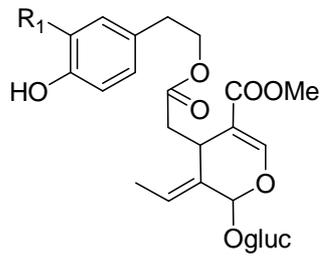
Structures of some lead compounds are given below-



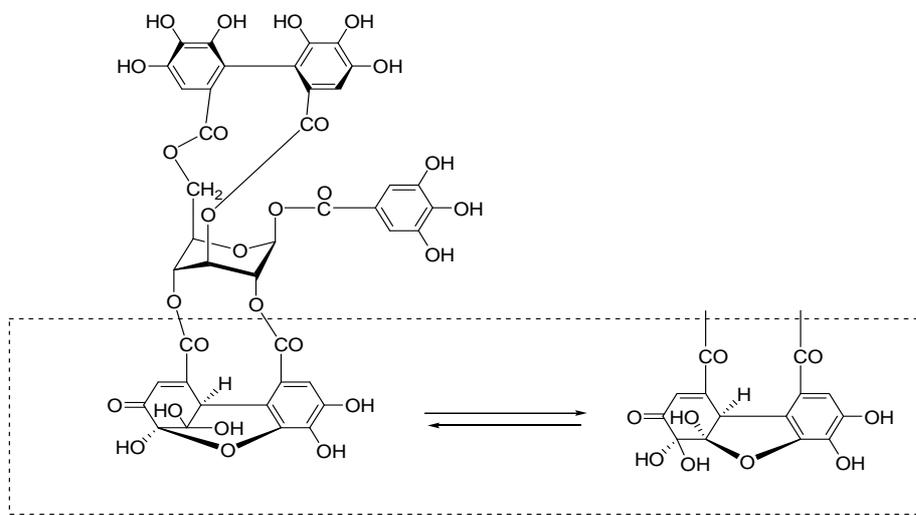
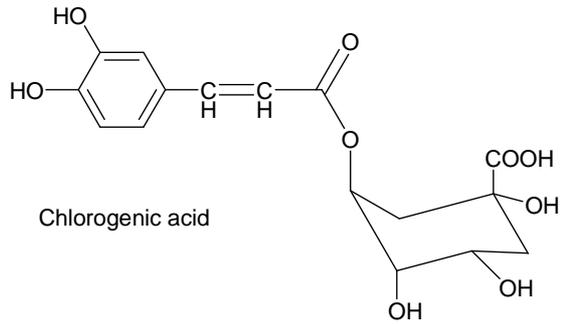
Isomartynoside ( $R_1=Me$ ,  $R_2=Me$ )



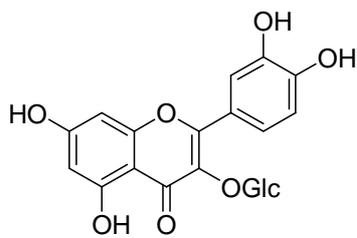
Apigenin 7-O-rutinoside ( $R_1=H$ ,  $R_2=gluc(6-1)rha$ )



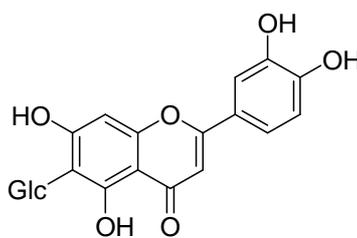
Ligstroside (R<sub>1</sub>=H) Oleuropein (R<sub>1</sub>=OH)



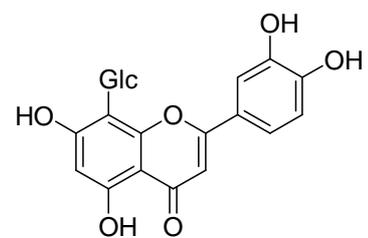
Geraniin



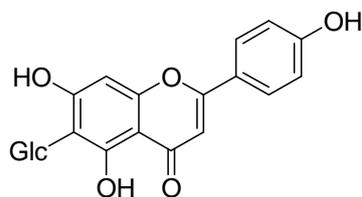
Isoquercetrin



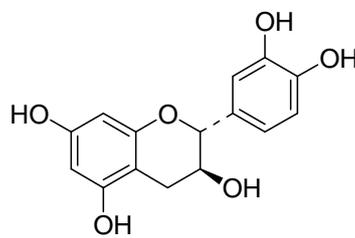
Isoorientin



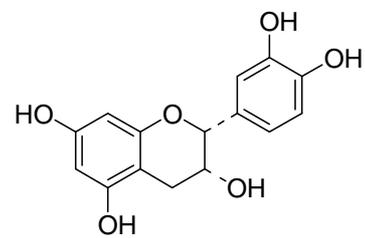
Orientin



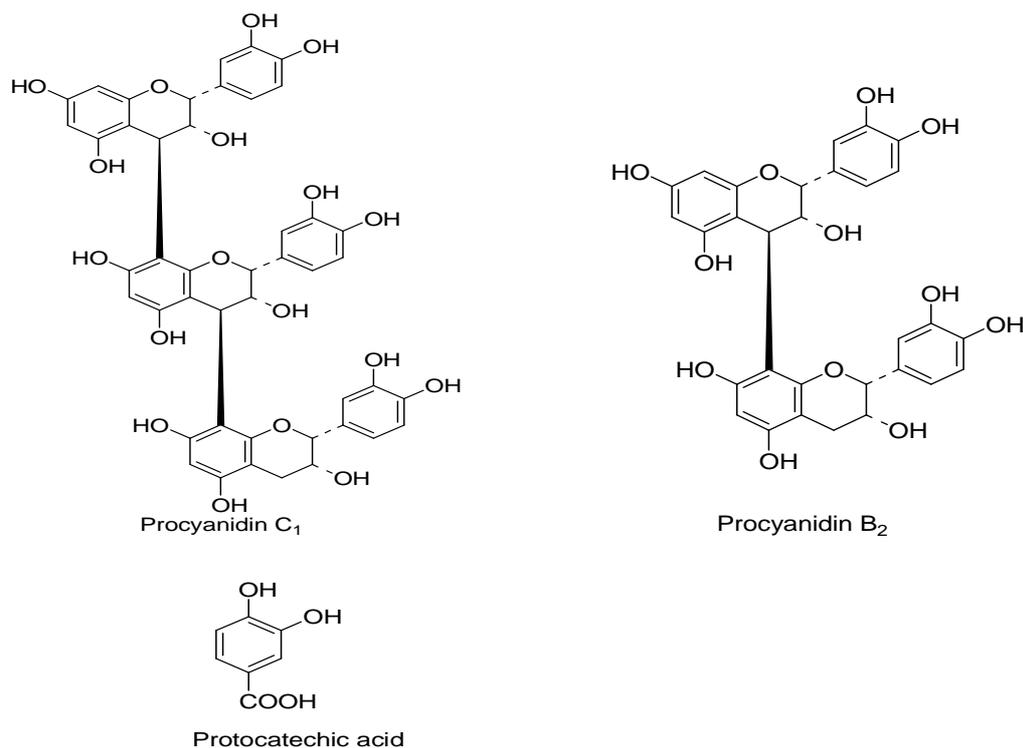
Isovitexin



(+)-Catechin



(-)-Epicatechin



### ACE inhibitor activity of isolated natural lead compounds of different plants

Lead Compound	Plant name	Inhibition (%)	Tested Concentration	Ref.
Acteoside	<i>A.distichum</i>	50	228 µg/mL	78
	<i>C. trichotomum</i>	50	376 ± 15.6µg/ml	37
Apigenin	<i>R.procumbens</i>	18	300µmol/L	80
Chlorogenic acid	<i>Cecropia hololeuca</i>	4	0.33 mg/ml	33
(+)-Catechin	<i>C. glaziovii</i>	16	0.33 mg/ml	33
(-)-Epicatechin	<i>C. pachystachya</i>	34	0.33 mg/ml	33
Geraniin	<i>P. niruri</i>	50	0.4 mM	59
	<i>Q.stenophylla</i>	50	4.0X 10 <sup>-4</sup> Mol/liter	58
Isoacteoside	<i>A.distichum</i>	50	290 µg/mL	78
Isoquercitrin	<i>C. glaziovii</i>	32	0.33 mg/ml	33
Isoorientin	<i>C. pachystachya</i>	48	0.33 mg/ml	33
Isovitexin	<i>C. pachystachya</i>	46	0.33 mg/ml	33
Luteolin	<i>R.procumbens</i>	55	300µmol/L	80
Orientin	<i>M. cecropioides</i>	20	0.33 mg/ml	33
Procyanidin B2	<i>C. glaziovii</i>	25	0.33 mg/ml	33
Procyanidin C1	<i>C. hololeuca</i>	45	0.33 mg/ml	33
Quercetin	<i>R.procumbens</i>	23	300µmol/L	80
Rutin	<i>A. distichum</i>	50	278 µg/mL	78
Sapogenin	<i>R.praertermisssa</i>	50.5	-	61
Vitexin	-	21	0.33 mg/ml	33

**Natural product:**

Synthetic ACE inhibitors are believed to have certain side effects, therefore, search for non-toxic, and safer, innovative and economical ACE inhibitors as alternatives to synthetic drugs is of great interest among researchers and many natural ACE inhibitors have been isolated from functional food and natural bio-resources. The peptides derived from food proteins are considered to be milder and safer compared with synthetic drugs. [18, 19] A detailed account from various natural products is described as below.

***Acaudina molpadioidea***

Protein from the *Acaudina molpadioidea* (sea cucumber) was hydrolyzed sequentially with bromelain and alcalase and fractionated into two ranges of molecular weight (PH-I, >2 kDa; PH-II, <2 kDa). It was found that *A. molpadioidea* at a dosage of 3µM/kg give the inhibitory activity by 3.5 times from IC<sub>50</sub> 15.9 to IC<sub>50</sub> 4.5 µM after incubation with gastrointestinal proteases [20, 21]

***Acetes chinensis***

Oligopeptide- enriched hydrolysates from *Acetes chinensis* by treatment with the protease from *Bacillus* sp. exhibited angiotensin-I-converting enzyme (ACE) inhibitory activity. The plant scaled hydrolysates caused reduce of 18.3–38.6 mmHg of the blood pressure of spontaneously hypertensive rats in dose-dependent manner in the range of 100–1200 mg/kg/day [22].

***Allium sativum***

Dipeptides from an aqueous extract of *Allium sativum* (Garlic) were identified as Ser-Tyr, Gly-Tyr, Phe-Tyr, Asn-Tyr, Ser-Phe, Gly-Phe, and Asn-Phe, with IC<sub>50</sub> values of 66.3, 72.1, 3.74, 32.6, 130.2, 277.9, and 46.3 µM, respectively. ACE inhibitory activity followed the order, with the N-terminal amino acid being Phe.Asn.Ser.Gly at the N terminal; the dipeptide Phe-Tyr was the most potent inhibitor of ACE. It is possible that these peptides cause ACE inhibition by chelating zinc, which is required for ACE activity. Daily use of garlic, may keep normal blood pressure from rising in some individuals [23].

***Amaranthus hypochondriacus***

*Amaranth hypochondriacus* has emerged as an attractive source of vegetal protein due to its high nutritional value. The occurrence of two inhibitory tetrapeptides, ALEP and VIKP in *A. hypochondriacus*, was predicted and experimentally validated by an *in vitro* ACE inhibition assay that showed IC<sub>50</sub> values of 6.32 µM and 175 µM, respectively [24].

***Biceps femoris***

Sarcoplasmic protein extracts from *Biceps femoris* (beef rump) were hydrolyzed (for 0, 4, 8, 12, and 24 h) with three enzymes or their paired combinations. The highest ACE inhibitory activity of enzyme hydrolysates resulted from 4 h incubation with enzymes or their paired combinations. The hexapeptide Val-Leu-Ala-Gln-Tyr-Lys, separated from the beef hydrolysates was started with valine. This peptide may be a potent ACE inhibitor which might perhaps be used to develop beef with a bioactive peptide to lower blood pressure [25].

**Bonito**

Dried bonito (Katsuobushi) has the inhibitory activity of the hydrolysates for angiotensin I-converting enzyme. Ile-Lys-Pro suppressed the hypertensive activity of angiotensin I. (26) Fragmentation of angiotensin-I by gastric juice was produced small peptides, such as IKYGD and IKWGD, had high ACE inhibitor activity. [27].

***Bos grunniens***

*Bos grunniens* (Yak) milk casein derived from Qula, alcalase hydrolysates has high and stable ACE-inhibiting activity, but yak milk casein itself showed very low antihypertensive activity. The molecular masses of the purified ACE inhibitors from yak casein hydrolysate were 550 Da and 566.4 Da, and their amino acid sequences were Pro-Pro-Glu-Ilu-Asn (PPEIN) and Pro-Leu-Pro-Leu-Leu (PLPLL), respectively. The IC<sub>50</sub> value of the peptides was 0.29 ± 0.01 mg/ml and 0.25 ± 0.01 mg/ml, respectively [28].

**Bovine**

Proteolysis of bovine plasma proteins can result in peptides with ACE inhibitory activity. The angiotensin I-converting enzyme (ACE) inhibiting activity of the hydrolyzed protein was assessed with hippuryl-His-Leu as the substrate. The amount of hippuric acid released, due to uninhibited ACE activity, was determined by high performance liquid chromatography. ACE inhibiting activity was found to increase with increasing degrees of hydrolysis; the 43% degrees of hydrolysate exhibited the highest activity and had an IC<sub>50</sub> of 1.08 mg/ml. The fraction that possessed the highest ACE inhibiting activity contained peptides with GYP, HL(I), HPY, HPGH, L(I)F, SPY, and YPH sequence motifs, Some of these moieties correspond to sequences found in bovine serum albumin, a potential source of ACE inhibiting peptides in bovine plasma [29]. Alcalase produced ACE inhibitory peptides from plasma proteins most efficiently and the Alcalase hydrolysate of albumin showed the highest activity (IC<sub>50</sub>=0.56 mg: ml). Albumin separated from bovine blood plasma is a promising protein source for the production of ACE inhibitory peptides as materials for antihypertensive functional foods [30].

***Brachionus rotundiformis***

Rotifers are the most commonly used marine zooplankton as live feed for fish larvae cultures. Rotifers are small size, rich nutrients, and an ideal feed source for large quantity fish cultivation. ACE inhibitory peptides were separated from rotifer hydrolysate prepared by Alcalase, α-chymotrypsin, Neutrase, papain, and trypsin. The Alcalase hydrolysate had the highest ACE inhibitory activity compared to the other hydrolysates. The IC<sub>50</sub> value of purified ACE inhibitory peptide was 9.64 μM, Amino acid sequence of the peptide was identified as Asp-Asp-Thr-Gly-His-Asp-Phe-Glu-Asp-Thr-Gly-Glu-Ala-Met, with a molecular weight 1538 Da [31].

***Brassica oleracea***

The water-soluble extract from *Brassica oleracea* (Broccoli) had 76.9% ACE inhibitory activity, while those of other organic solvent extracts showed lower ACE inhibitory activities. The purified ACE inhibitory peptide was identified to be a tripeptide, Tyr-Pro-Lys, having an IC<sub>50</sub> value of 10.5 μg protein/ml [32].

***Cecropia hololeuca***

The ethanolic extract of *Cecropia hololeuca* leaves showed an ACE-inhibition of  $40 \pm 4\%$  at a concentration of 0.33 mg/ml. Extracts showed content in procyanidins of about 11 %. [33].

**Chicken egg yolks**

Oligopeptides of 1 KDa or less were obtained by hydrolysis of chicken egg yolks with a crude enzyme had an inhibitory action on the activity of angiotensin I-converting enzyme *in vitro*. Oligopeptides extracted from hen's egg yolks could potentially suppress the development of hypertension in SHR, and this effect might be induced by the inhibition of ACE activity. Oligopeptides could be used as a physiologically functional food to control blood pressure in patients with essential hypertension [34].

***Chlorella vulgaris***

A peptide with angiotensin I-converting enzyme (ACE) inhibitory activity was isolated from the pepsin hydrolysate of algae protein waste, a mass-produced industrial by-product of an algae essence from microalgae, *Chlorella vulgaris*. Edman degradation revealed its amino acid sequence to be Val-Glu-Cys-Tyr-Gly-Pro-Asn-Arg-Pro-Gln-Phe. Inhibitory kinetics revealed a non-competitive binding mode with  $IC_{50}$  value against ACE of  $29.6\mu M$  [17].

***Chrysanthemum boreale***

Crude water extract of the flowers of *Chrysanthemum boreale* was prepared by steeping in water at  $95\text{ }^{\circ}C$  for 2 h, followed by centrifugation at  $8000 \times g$  for 30 min. Crude extract was then filtered using YM-3 and YM-1 membranes [35].

***Cicer arietinum***

*Cicer arietinum* (Chickpea) is the third most important grain legume, having Legumin as main storage protein in chickpea. Treatment of legumin with alcalase yielded a hydrolysate that inhibited the angiotensin I converting enzyme with an  $IC_{50}$  of 0.18 mg/ml. Fractionation of this hydrolysate by reverse phase chromatography afforded six inhibitory peptides with  $IC_{50}$  values ranging from 0.011 to 0.021 mg/ml. All these peptides contain the amino acid methionine and are also rich in other hydrophobic amino acids. Hydrolysates of chickpea legumin obtained by treatment with alcalase are a good source of peptides with angiotensin I-converting enzyme inhibitory activity [36].

***Clerodendron trichotomum***

Bioassay-guided fractionation and purification of the EtOAc-soluble extract of *Clerodendron trichotomum* afforded acteoside, leucosceptoside A, martynoside, acteoside isomer, and isomartynoside. The angiotensin converting enzyme (ACE) activities were significantly inhibited by the addition of these phenylpropanoid glycosides in a dose-dependent manner of which  $IC_{50}$  values were  $373 \pm 9.3\mu g/ml$ ,  $423 \pm 18.8\mu g/ml$ ,  $524 \pm 28.1\mu g/ml$ ,  $376 \pm 15.6\mu g/ml$ ,  $505 \pm 26.7\mu g/ml$ , respectively. The antihypertensive effect of *C. trichotomum* may be, at least in part, due to ACE inhibitory effect of phenylpropanoid glycosides. The methanol extract of the stem of *C. trichotomum* was found to possess a relatively high ACE inhibition ( $IC_{50} = 568.5 \pm 12.4\mu g/ml$ ) [37].

***Corbicula fluminea***

*Corbicula fluminea* (Freshwater clam) is hydrolyzed by Protamex for 5 h showed an ACE IC<sub>50</sub> value being 0.043 mg/ml. The systolic blood pressure and diastolic blood pressure of spontaneously hypertensive rats fed the hydrolysate (peptide concentration 5 mg/ml) for 8 weeks were significantly reduced by 22.0 and 13.2 mmHg, respectively. Two inhibitory peptides were isolated from the hydrolysate showing high ACE inhibitory activities and their amino acid sequences were Val-Lys-Pro and Val-Lys-Lys, which demonstrated ACE inhibitory activity *in vitro* and antihypertensive activity *in vivo*. The inhibition was likely caused by binding of the tripeptides on both the active site and non-catalytic site of the ACE. ACE inhibitor derived from freshwater clam hydrolysates could be utilized to develop functional foods for prevention of hypertension [38].

***Crassostrea gigas***

ACE inhibitory activity of fermented oyster sauce was investigated, and the IC<sub>50</sub> value was determined to be 2.45 mg/ml. oyster sauce may be useful as functional food for the maintenance of blood pressure within the normal range. ACE inhibitor derived from fermented oyster sauce could be utilized to develop physiologically functional foods [39].

***Crassostrea talienwhanensis***

A purified peptide with sequence Val-Val-Tyr-Pro-Trp-Thr-Gln-Arg-Phe (VVYPWTQRF) was firstly isolated from *Crassostrea talienwhanensis* (oyster protein) hydrolysate and its ACE inhibitory activity was determined with IC<sub>50</sub> value of 66μ mol/L *in vitro*. The oyster protein hydrolysate (fraction II), prepared by pepsin treatment firstly exhibited antihypertensive activity when it was orally administered to spontaneously hypertensive rat at a dose of 20mg/kg. The hydrolysate from oyster proteins prepared by pepsin treatment could serve as a source of peptides with antihypertensive activity. Peptide derived from oyster proteins could be utilized as nutraceuticals and pharmaceuticals [40].

***Cryptomeria japonica***

The ethanol extract from outer bark of *Cryptomeria japonica* (Japanese cedar) showed the highest inhibitory activity (IC<sub>50</sub> is 16μg/ml) among 24 extracts prepared from roots, leaves, heartwood, sapwood, inner bark, and outer bark by successive extraction with four solvents. The fractionation of the outer bark ethanol extract followed by the bioassay resulted in the isolation of two strong ACE inhibitors, catechin and dimeric procyanidin B3. The bioassay of three flavan-3-ols including (+)-catechin and six flavones revealed that most of these compounds have high ACE inhibitory activity [41].

***Ctenopharyngodon idella***

*Ctenopharyngodon idella* (Grass carp fish) is one of the largest members of the minnow family, which inhabits fresh warm water. MAR DA201-C was thus a good material for desalting and enhancing peptides with *in vitro* ACE inhibitory properties. Grass carp fish scale peptide had higher ACE inhibitory capabilities *in vitro*. The lowest concentration at which the eluted fraction possessed half of its original ACE activity (IC<sub>50</sub>) was 0.13 mg/ml. The results indicated that fish scales are a good source for peptides with higher *in vitro* ACE inhibitory activity, which could be a potential value-added product in the market [8].

***Cuscuta japonica* Choisy**

EtOAc-soluble extract of *Cuscuta japonica* afforded 3, 5-Di-*O*-caffeoylquinic acid, methyl 3, 5-Di-*O*-caffeoylquinic acid, 3, 4-Di-*O*-caffeoylquinic acid, and methyl 3, 4-Di-*O*-caffeoylquinic acid. Compounds inhibited the angiotensin I converting enzyme activity in a dose-dependent manner. These compounds showed the 50% inhibitory concentration values of 596, 483, 534, and 460  $\mu$ M, respectively [42].

**Douchi**

Douchi is a traditional fermented soybean product originating in China, produces angiotensin I-converting enzyme (ACE) inhibitors with the potential to lower blood pressure. The ACE inhibitory activities of douchi qu pure-cultured by *Aspergillus Egyptiacus* for 48 h, and 72 h were compared with douchi secondary-fermented for 15 d. Douchi qu, fermented by *A. egypticus*, showed clear ACE inhibitory activity, which was enhanced following primary and secondary fermentation. The ACE inhibitors in 48 h-fermented douchi qu were fractionated into four major peaks by gel filtration chromatography on Sephadex G-25. Peak 2, which had the highest activity, had only one peptide, composed of phenylalanine, isoleucine and glycine with a ratio of 1:2:5 [43].

**Fermented milk proteins**

Fermented milk product with the biologically active peptides valyl-prolyl-proline (Val-Pro-Pro) and isoleucyl-prolyl-proline (Ile-Pro-Pro) was shown to lower blood pressure in spontaneously hypertensive rats. Two other peptides (Tyr-Pro and Lys-Val-Leu-Pro-Val-Pro-Gln) that were purified and characterized from fermented milk were also shown to have ACE-inhibitory activity in spontaneously hypertensive rats. *L. helveticus* LBK-16H fermented milk, in normal daily use, has a blood pressure lowering effect in hypertensive subjects and is thus potentially useful in the dietary treatment of hypertension [44].

Fermented milk whey product inhibited ACE *in vitro*. The bioactivity was contributed mainly by peptides of Gly-Thr-Trp and Gly-Val-Trp, and confirmed *in vivo* by preventing hypertension in SHR (reduction in SBP by 22.0mm Hg) after 8 weeks of oral administration of diluted whey (peptide concentration 5mg/mL) from the 30 h fermentation. The IC<sub>50</sub> values were 464.4 and 240.0 $\mu$ M, respectively. Fermented milk whey was expected to be a useful ingredient in physiologically functional foods for the prevention of hypertension [45].

***Fagopyrum esculentum***

*Fagopyrum esculentum* (Buckwheat) is an herbaceous plant, is recognized as a valuable source of so-called “functional food”. The ACE inhibitor was identified to be a tripeptide, Gly-Pro-Pro, having IC<sub>50</sub> value of 6.25  $\mu$ g protein/ml. The ACE inhibitory peptides with high activities could be derived from protein of *F. esculentum*, which have been traditionally used as a Korean medicine treatment for patients having hypertension. The ACE inhibitory peptides of *F. esculentum* may be useful as a functional food ingredient with anti-hypertensive property [46].

***Graptopetalum paraguayense***

Water, 50% ethanolic and 95% ethanolic extracts from *Graptopetalum. paraguayense* showed potent inhibitory effects on ACE. ACE inhibitory activities of all the tested extracts increased with the increase of their concentrations. The ACE inhibitions of the tested extracts of G.

paraguayense were significantly reduced after the addition of 1.5 mM ZnCl<sub>2</sub>, suggesting the inhibitory action of the extracts may have resulted from the chelation of the ACE zinc cofactor. 50% ethanolic and 95% ethanolic extracts from *G. paraguayense* exhibited the more effective ACE inhibitory activity than the water extracts of *G. paraguayense*. [47].

### ***Grifola frondosa***

The most potent ACE inhibitory activity (58.7%) was detected in cold water extract of *Grifola frondosa*, with an IC<sub>50</sub> of 0.95mg. The ACE inhibitory activities of cold and hot water extracts increased as the extract time increased, but decreased slightly 15h and 90 min, respectively, after extraction. A novel ACE inhibitor from *G. frondosa*, a peptide (Val-Ile-Glu-Lys-Tyr-Pro) composed of a hydrophobic amino acid at the amino terminal, a basic amino acid residue at the centre, and proline at the carboxyl-terminal. After the purification of ACE inhibitory peptides with acetone fractionation and column chromatography, obtained an active fraction with an IC<sub>50</sub> of 0.13mg and a yield of 0.7%. [48].

### ***Gynura procumbens***

A partially purified fraction of the leaves of *Gynura procumbens* is able to cause a dose-dependent fall in the both spontaneously hypertensive rats and normotensive Wistar-Kyoto rats, with an ED<sub>50</sub> of 1.09 and 1.05 mg/kg, respectively (p <0.01). A partially purified aqueous fraction at 10 mg/kg strongly inhibited the angiotensin I-induced rise in mean arterial pressure (p < 0.01). Hypotensive effect of *G. procumbens* may be due, in part, to the glycoconjugated or peptidal substances found in a partially purified fraction that exhibit an inhibitory effect on ACE [49].

### ***Helianthus annuus***

*Helianthus annuus* (Sunflower) is one of the most important oil seed crops in the world. Reverse-phase HPLC fractionation of this product yields several fractions with IC<sub>50</sub> one order of magnitude higher than those purified by reverse-phase HPLC following gel filtration chromatography, showing that affinity chromatography is much more effective than gel filtration chromatography as a first step for purification of ACE inhibitory peptides. (50)

### ***Ligustrum vulgare***

At a concentration of 100µg/ml the ethyl acetate extracts of *Ligustrum vulgare* (Oleaceae) showed the highest inhibitory activity against ACE enzyme. The bioguided fractionation of the leaves extract led to the isolation of two iridoids which were identified as oleuropein and ligstroside aglycones. Both compounds are dual ACE/NEP inhibitors with IC<sub>50</sub> of 20 and 25µM for ACE and IC<sub>50</sub> of 35 and 75µM for NEP, respectively [51].

### ***Limanda aspera***

*Limanda aspera* (Yellowfin sole frame) protein, which is normally discarded as industrial waste in the process of fish manufacture. The total solid mass of the frame consists of considerable amounts of protein, which can be used as potential bioactive substances. The ACE inhibitory peptide with a molecular mass of 1.3 kDa consisted of 11 amino acids, Met-Ile-Phe-Pro-Gly-Ala-Gly-Gly-Pro-Glu-Leu, and its IC<sub>50</sub> value was 28.7µg/ml [52].

***Morinda citrifolia***

Noni is the name for the fruit of the *Morinda citrifolia* tree. Noni juice exhibited strong ACE inhibitory activity. The inhibitory effect of juice from ripe fruit is stronger than that from green fruit. Single oral administration of the juice reduces the systolic blood pressure spontaneously in hypertensive male rats. Scopoletin is one of the most important phenolic compounds in noni juice. Scopoletin, which has been claimed to reduce blood pressure through a vasodilating effect, might have an ACE inhibitory effect. Some of flavonoids and condensed tannins, such as vitexin, isovitexin, (+)-catechin, isoquercitrin and (-)-epicatechin, have been found to exhibit ACE inhibitory activity [54].

***Musanga cecropioides***

The methanolic extract of the leaves of *Musanga cecropioides* showed an ACE-inhibition of 100% at 0.33 mg/ml. A higher procyanidins content of 13% was determined in *M. cecropioides* leaves [33].

***Mytilus edulis***

The IC<sub>50</sub> value of fermented blue mussel sauce for ACE activity was 1.01 mg/ml. The IC<sub>50</sub> value of purified ACE inhibitory peptide was 19.34 μg/ml. The N-terminal amino acid sequence of the purified ACE inhibitory peptide is EVMAGNLYPG. Fermented blue mussel sauce may have beneficial effects on hypertension. ACE inhibitory peptide derived from fermented blue mussel sauce could be utilized to develop potentially functional foods [55]

***Panax ginseng***

The effects of the *Panax ginseng* extract G115 was investigated on angiotensin-converting enzyme (ACE) activity and nitric oxide (NO) in cultured human endothelial cells from umbilical veins and bovine mesenteric arteries. In cultured human endothelial cells from umbilical veins, ACE activity was significantly reduced after 10 min incubation with aqueous extract of ginseng 5.0 and 10 mg/ml. Angiotensin I-induced contraction of bovine mesenteric arteries was significantly attenuated by 0.1 and 0.5 mg/ml ginseng. Extract of *P. ginseng* (G115) inhibits ACE activity. [57].

***Phyllanthus niruri***

The n-BuOH extract of the *Phyllanthus niruri* was found to have higher activity than the EtOH extract. IC<sub>50</sub> for ACE activity of geraniin, the most active of the isolated compounds, of the *P. niruri* was  $4.0 \times 10^{-4}$  mol/liter. Geraniin might interact with the zinc atom in ACE [58].

***Phyllanthus urinaria***

Geraniin, the hydrolysable tannin, was purified from the 70% aqueous acetone extracts of *Phyllanthus urinaria* (Pearls under the leaves" in Chinese). Geraniin also showed dose-dependent inhibitory activities against angiotensin converting enzyme (ACE, IC<sub>50</sub> were 13.22 μM). The geraniin showed antihypertensive activity in lowering systolic blood pressure (SBP) and diastolic blood pressure (DBP). [59].

**Porcine skeletal muscle**

ACE inhibitory activities derived from the digests of the water-insoluble protein fraction prepared from porcine skeletal muscle, thermolysin digest demonstrated the highest activity.

Two ACE inhibitory peptides were purified from thermolysin digest of myosin. The sequences of these inhibitory peptides, named myopentapeptides A and B, were Met-Asn-Pro-Pro-Lys and Ile-Thr-Thr-Asn-Pro. The concentrations of the peptides showing 50% inhibition values ( $IC_{50}$ ) of ACE were 945.5 and 549.0  $\mu$ M, respectively. Also, six tripeptides, Met-Asn-Pro, Asn-Pro-Pro, Pro-Pro-Lys, Ile-Thr-Thr, Thr-Thr-Asn, and Thr-Asn-Pro, which have parts of the sequences of the myopentapeptides demonstrated activity. Their  $IC_{50}$  values were 66.6, 290.5, >1000, 678.2, 672.7, and 207.4 $\mu$ M, respectively. Porcine muscle proteins could be utilized to develop physiologically functional foods [9].

#### ***Rana catesbeiana***

Alcalase-proteolytic hydrolysates of *R. catesbeiana* showed the highest ACE-I inhibitory activity. Gly-Ala-Ala-Glu-Leu-Pro-Cys-Ser-Ala-Asp-Trp-Trp (Mw: 1.3 kDa) was isolated from *R. catesbeiana* muscle hydrolysates degraded by Alcalase ( $IC_{50}$  value of 0.95  $\mu$ M). Peptide derived from bullfrog muscle protein could be applied into nutraceuticals and pharmaceuticals through representation of phenol-type activity by enzymatic modification [60].

#### ***Ruellia praetermissa***

The aqueous extract (89.65%) was found to be the most active, possibly due to saponigenins. lupeol is one of the constituents responsible for the effect in the *n*-hexane extract, and flavonoids (luteolin and apigenin) might be responsible for the activity in the methanol and aqueous extracts. This activity suggests a cardiovascular effect of the aqueous extract of *R. praetermissa* [61].

#### ***Salsola oppositifolia***

Ethyl acetate extracts of *Salsola oppositifolia* revealed interesting inhibition on ACE enzyme activity. *In vitro* bio-assay based on the measured enzymatic cleavage of the chromophore-fluorophore-labelled substrate dansyltriglycine into dansylglycine and diglycine by angiotensin converting enzyme (ACE) was performed [62].

#### **Sardine muscle**

Hydrolyzates which inhibit the angiotensin I-converting enzyme (ACE) were prepared from sardine muscle by *Bacillus licheniformis* alkaline protease. The most potent activity was obtained when eluting with 10% ethanol ( $IC_{50}$  = 0.015 mg protein/mL). This fraction was apparently rich in acidic amino acids, poor in hydrophobic ones, and effective for use as powerful ACE inhibitory activity [63].

#### ***Sardinella aurita***

The alkaline protease extract from the viscera of *Sardinella aurita* (sardinelle) produced hydrolysate with the highest ACE inhibitory activity ( $63.2 \pm 1.5\%$  at 2 mg/ml). The  $IC_{50}$  values for ACE inhibitory activities of sardinelle by-products protein hydrolysates was  $1.2 \pm 0.09$  mg/ml. The amino acid analysis having ACE inhibitory activity was rich in phenylalanine, arginine, glycine, leucine, methionine, histidine and tyrosine. ACE inhibitors derived from sardinelle by-products could be utilized to develop functional foods for prevention of hypertension [64].

**Sea bream scales**

Oral administration of 300 mg of the peptides (kg of body weight)<sup>-1</sup> d<sup>-1</sup> of hydrolysate of sea bream scales was shown to decrease blood pressure significantly ( $P < 0.05$ ). Four inhibitory peptides showing high ACE inhibitory activities were isolated from the hydrolysate and their amino acid sequences were determined to be Gly-Tyr, Val-Tyr, Gly-Phe and Val-Ile-Tyr. The hydrolysate produced from sea bream scale demonstrated ACE inhibitory activity *in vitro* and antihypertensive activity *in vivo*, this hydrolysate product could be used as food material and as a treatment of hypertension [65].

**Shark meat**

Cys-Phe, Glu-Tyr, Met-Phe and Phe-Glu. Cys-Phe, Glu-Tyr and Phe-Glu sequences were conformed to be novel ACE inhibitory peptides from shark meat hydrolysate with IC<sub>50</sub> values of 1.96, 2.68 and 1.45 μM, respectively. They may have potential in the treatment of hypertension or in clinical nutrition. Shark meat hydrolysate obtained with protease SM98011 digestion showed high angiotensin-I-converting enzyme (ACE) inhibitory activity, with an IC<sub>50</sub> value of 0.4 mg/mL [66].

***Spodoptera littoralis***

An edible insect *Spodoptera littoralis* (Lepidoptera), can be used as a source of ACE inhibitory peptides after hydrolysis with enzymes. A new ACE inhibitory tripeptide, Ala-Val-Phe, was purified from this hydrolysate and assay revealed an IC<sub>50</sub> value of 2123 μM. This peptide could be applied as ingredient in functional and novel foods, dietary supplements or even pharmaceuticals as an antihypertensive agent [67].

***Sorghum bicolor***

*Sorghum bicolor* (Sorghum) is an important food for people living in the semi-arid tropical areas of Africa and Asia. Hydrolysis of sorghum kafirin using the protease chymotrypsin yielded a hydrolysate rich in peptides with ACE inhibitory activity. The hydrolysates alternatively could be used as a starting material for antihypertensive drugs as ACE inhibitors [19].

**Soy protein**

The most active hydrolysate was obtained by Alcalase hydrolysis of isolated soy protein. By using a 10 kDa molecular weight cut-offs membrane, the ACE inhibitory activity (IC<sub>50</sub>) of the hydrolysate decreased from 0.688 to 0.078 mg protein/ml. The lower the IC<sub>50</sub> represents the higher the ACE inhibitory activity [68].

ACE inhibitory peptides (WL and IFL) were isolated recently from tofuyo, which is a soybean curd fermented by fungi such as *Monascus* and *Aspergillus*. Soybean proteins, β-conglycinin and glycinin were hydrolysed by an acid proteinase from *Monascus purpureus*. The IC<sub>50</sub> values of the β-conglycinin and glycinin hydrolysates were determined as 0.126 mg/ml and 0.148 mg/ml, respectively. ACE inhibitory peptides isolated from the β-conglycinin hydrolysate were identified as LAIPVNKP (IC<sub>50</sub> = 70 μM) and LPHF (670 μM), and those from the glycinin hydrolysate as SPYP (850 μM) and WL (65 μM). The inhibitory activity of SPYP markedly increased after successive digestion by pepsin, chymotrypsin and trypsin *in vitro* [69].

**Tofuyo**

Tofuyo is a soybean curd fermented by fungi such as *Monascus* and *Aspergillus*, which has angiotensin I converting enzyme (ACE) inhibitory activity *in vitro*. ACE inhibitory peptides Ile-Phe-Leu and Trp-Leu were isolated from Tofuyo [70].

***Theragra chalcogramma***

Proteolytic digestion of gelatin extracts from *Theragra chalcogramma* (Alaska Pollack) skin brings about a high angiotensin I converting enzyme (ACE) inhibitory activity. The isolated peptides were composed of Gly-Pro-Leu and Gly-Pro-Met and showed IC<sub>50</sub> values of 2.6 and 17.13 μM, respectively. Gly-Pro-Leu would be useful as a new antihypertensive agent [71].

***Tricholoma giganteum***

The ACE inhibitory activity (IC<sub>50</sub>: 0.31 mg) was obtained when the fruiting body of *T. giganteum* was extracted with distilled water at 30°C for 3 h. The ACE inhibitory peptide was a novel tripeptide, showing peptide sequences, as Gly-Glu-Pro. ACE inhibitor from *T. giganteum* showed a clear antihypertensive effect in spontaneously hypertensive rats, at a dosage of 1mg/kg. Therefore, the ACE inhibitory peptide from *T. giganteum* will be very useful in the preparation of antihypertensive drugs and functional foods [72].

***Tulbaghia violacea***

*Tulbaghia violacea* (Wild garlic), showed more than 50% inhibition in organic (methanol) and aqueous extract preparations. *T. violacea* has shown properties related to lowering blood pressure [67].

**Tuna frame protein**

Tuna frame proteins are usually discarded as processing waste or used for animal feed because of its poor functional properties. A potent ACE inhibitory peptide from tuna frame protein (PTFP), which was composed of 21 amino acids, Gly-Asp-Leu-Gly-Lys-Thr-Thr-Thr-Val-Ser-Asn-Trp-Ser-Pro-Pro-Lys-Try-Lys-Asp-Thr-Pro (MW: 2,482 Da, IC<sub>50</sub>: 11.28 μM), was isolated. Oral administration of peptide from tuna frame protein can decrease systolic blood pressure significantly (P < 0.01). The peptide from tuna frame protein would be a beneficial ingredient for nutraceuticals and pharmaceuticals against hypertension and its related diseases [18].

A novel inhibitor of angiotensin-converting enzyme (ACE) was discovered and isolated in a pure form from acid extract of tuna muscle by successive column chromatography and HPLC. The final preparation showed IC<sub>50</sub> values of 1 and 2 μM for ACEs from bovine and rabbit lungs, respectively. The amino acid sequence of the inhibitor was Pro-Thr-His-Ile-Lys-Trp-Gly-Asp, by the Edman procedure and carboxypeptidase digestion. This peroxide may be of interest in treatment of hypertension [73].

***Undaria pinnatifida***

*Undaria pinnatifida* (Wakame) is the most popular edible sea weed in Japan. A peptide fraction having activity against angiotensin-I converting enzyme (ACE) was separated from the peptic digest of protein prepared from wakame, These tetrapeptides were identified by sequence analysis and fast atom bombardment mass spectrometry as Ala-Ile-Tyr-Lys (IC<sub>50</sub>=213 μM), Tyr-

Lys-Tyr-Tyr (64.2  $\mu\text{M}$ ), Lys-Phe-Tyr-Gly (90.5  $\mu\text{M}$ ), and Tyr-Asn-Lys-Leu (21  $\mu\text{M}$ ). These tetrapeptides in peptic digest of wakame could be responsible for lowering blood pressure [74].

#### **Wheat germ**

Wheat germ hydrolyzate with the most potent ACE inhibitory activity was obtained by 0.5 wt.% 8 h *Bacillus licheniformis* alkaline protease hydrolysis after 3.0 wt.%-3 h  $\alpha$ -amylase treatment of defatted WG ( $\text{IC}_{50}$  = 0.37 mg protein/ml). Powerful ACE inhibitory activity ( $\text{IC}_{50}$  = 0.48  $\mu\text{M}$ ), Ile-Val-Tyr was identified as a main contributor to the ACE inhibition of the hydrolyzate [76]

#### **Wines**

Wine is a product rich in phenols, peptides. The activity determined ranges from 10.3% to 95.4%, with significantly higher mean values in red wines than in the other wines. The majority amino acids detected were also Asx and Glx, Val, Ser, Lys, Gly and  $\alpha$ -Ala. This amino acid frequently forms part of peptides with inhibitory activity of angiotensin-converting enzyme in hydrolyzed milk proteins and in sake lees [77].

#### **Natural lead compounds**

A lot of plant extracts and isolated compounds such as terpenoids, alkaloids, tannins, proanthocyanidins and flavonoids have been reported as ACE inhibitors. Elbl and Wagner supposed that there is possibility of chelate complexes formation with the zinc atom within the active centre of the ACE. This chelate could be done between heterocyclic oxygen and a phenolic hydroxyl group in its vicinity [62].

Peptides derived from the major whey proteins, i. e.  $\alpha$ -lactalbumin ( $\alpha$ -la) and  $\beta$ -lactoglobulin ( $\beta$ -lg) in addition to bovine serum albumin, inhibit ACE. The most potent lactokin in reported to date, ( $\beta$ -lg f (142-148)), has an ACE  $\text{IC}_{50}$  of 42.6  $\mu\text{mol/l}$  [78].

Fractionation of the n-BuOH extract of *Abeliophyllum distichum* afforded acteoside, isoacteoside and rutin. Compounds showed the 50% inhibitory concentration values of 228  $\mu\text{g/mL}$ , 290  $\mu\text{g/mL}$  and 278  $\mu\text{g/mL}$  [53]. Flavonoids and proanthocyanidins demonstrated inhibitory activity at 0.33 mg/ml [56].

*Salsola tragus* is reported to contain tetrahydroisoquinoline alkaloids such as salsoline and salsolidine, fatty acids, and flavonoids such as isorhamnetin-3-O-glucoside and isorhamnetin-3-O-rutinoside. (62) Almost all ACE inhibitors were peptides, except *Ganaderma lucidum*, which was a triterpene [72].

EtOAc-soluble extract of *Clerodendron trichotomum* afforded acteoside, leucosceptoside A, martynoside, acteoside isomer, and isomartynoside. Among the isolated compounds, acteoside and the acteoside isomer showed the most potent ACE inhibition due to the generation of chelate complexes with zinc ions  $\text{IC}_{50}$  values of  $373 \pm 9.3 \mu\text{g/ml}$  and  $376 \pm 15.6 \mu\text{g/ml}$  respectively [37]. Purified geraniin exhibited antioxidant activities, semicarbazide-sensitive amine oxidase and ACE inhibitory activities, and antihypertensive effects on spontaneously hypertensive rats. Geraniin isolated from *P. niruri* has been reported to have ACE inhibitory activity using hippuryl-L-His-His-Leu as a substrate and the  $\text{IC}_{50}$  was 0.4 mM [59].

Uchida et al. isolated some condensed tannins from “rhei rhizoma” as ACE inhibitors, and Kameda et al. Reported (+)-catechin as the inhibitor from *Quercus stenophylla*. IC<sub>50</sub> for ACE activity of geraniin, the most active of the isolated compounds, was 4.0X 10<sup>-4</sup> mol/liter [81].

The EtOAc, MeOH and the aqueous extracts of *Ruellia praertermissa* have apigenin as one of the flavone aglycones, which shows an antihypertensive effect by both affecting ACE and calcium channel blocking activity [61].

The trimeric procyanidin C1 was more active than the dimeric procyanidin B2 (45 ± 2% and 25 ± 5% inhibition, respectively). ACE-inhibiting activity was reported for isolated compounds such as vitexin, isovitexin and (+)-catechin, isoquercitrin and procyanidin B2. Orientin, isoorientin, (-) - epicatechin, and procyanidin C1 which are reported for the first time with an ACE-inhibitory activity [36].

Aglycones of secoiridoids oleuropein and ligstroside are responsible for the inhibitory activity of *Ligustrum* extracts on metalloproteinases. The inhibition of ACE and NEP may result in a decrease of blood pressure and an increase of diuresis [51].

Compounds apigenin, luteolin and quercetin were ACE-inhibitory active compound of plant *Rostellularia procumbens* with inhibitory ratios of 18%, 55% and 23%, respectively at maximal concentration 300 μmol/L. Compound quercetin could inhibit ACE with inhibitory ratio of 31.32%, when 88.7 μmol/kg was administered in vitro, which was similar to that of captopril. Compound quercetin showed ACE-inhibitory activity with IC<sub>50</sub> 226 μmol/L [76].

### **Current aspect and future trends**

The ACE inhibitors now constitute one of the most important classes of cardiovascular drugs. The developments of specific agents that interfere with the renin-angiotensin system have defined the contribution of this system to blood pressure regulation and to the pathogenesis of hypertension, congestive heart failure, and chronic renal failure [79]. A major breakthrough in the treatment of blood pressure was the design of the captopril, which has spawned an entire industry. [80] To achieve good oral bioavailability, most of the inhibitors (except captopril and lisinopril) are synthesized as ester prodrug [82] but it also opened the door to the design of new and more targeted ACE inhibitors [81].

Although several synthetic ACE inhibitors are now widely available for use as antihypertensive drugs, they have been known to cause some undesirable side effects such as postural hypotension, cough, renal failure and angioedema. Currently, many studies are being done to search for more suitable antihypertensive agents, including ACE inhibitors, from natural products. Findings from these studies may open up the possibilities of more alternatives with ACE inhibitory effects with better drug profiles and less adverse side effects.

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