J Wood Sci (1998) 44:463–468
© The Japan Wood Research Society 1998

ORIGINAL ARTICLE

Yuji Tsutsumi · Ayako Shimada · Akiko Miyano
Tomoaki Nishida · Tohru Mitsunaga

In vitro screening of angiotensin I-converting enzyme inhibitors from Japanese cedar (*Cryptomeria japonica*)

Received: November 20, 1997 / Accepted: May 19, 1998

Abstract Screening and isolation of angiotensin I-converting enzyme (ACE) inhibitors from Japanese cedar (*Cryptomeria japonica*) based on the in vitro ACE inhibitory assay were attempted. The ethanol extract from outer bark showed the highest inhibitory activity (IC_{50} is 16 μg/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. The results suggest that the phenolic hydroxyl group at the C7 position and heterocyclic oxygen atom of these compounds are important for expressing the inhibitory activity.

Key words ACE inhibitor · *Cryptomeria japonica* · Catechin · Procyanidin B3 · Flavonoid

Introduction

Japanese cedar (*Cryptomeria japonica* D. Don) is one of the most abundant planted woody species in Japan. The wood of Japanese cedar is traditionally used for poles, shingles, and lumber for exterior construction because of its durability. To develop a new utilization method for this species, we have focused on the biological or pharmacological activity of Japanese cedar extract. Previously we reported that sequirin-C isolated from its heartwood has high superoxide dismutase (SOD) mimic and tyrosinase inhibitory activities.[1] In this study, we investigated angiotensin I-converting enzyme (ACE, peptidyl dipeptide hydrolase EC. 3. 4. 15. 1) inhibitors from Japanese cedar.

It is well known that ACE inhibition is one of the effective treatments for hypertension. ACE catalyzes the formation of octapeptide angiotensin II, a strong pressor, from the decapeptide angiotensin I. The synthetic ACE inhibitors captopril and its analogs were originally developed from snake (*Bothrops jararaca*) venom.[2] Their key feature was a prominent binding moiety to zinc, which is the active site of ACE.[3] Some medicinal plants were submitted to ACE inhibitory screening,[4] and the isolation of active principles from medicinal plants were reported.[5–10] In such studies, some plant peptides[5] were shown to have ACE inhibitory activity in the same manner as captopril. On the other hand, some flavonoids and procyanidins from *Lespedeza capitata*[6,7] and tetrahydroxyxanthones from *Tripterospermum lanceolatum*[8] were also reported as ACE inhibitors. These plant metabolites that originate from the phenylpropanoid pathway generally have phenolic hydroxyl group(s) (sometimes methylated) in their molecules. These hydroxyl groups have a potential to form chelate complexes with zinc in ACE as well as peptidic ACE inhibitors.

Japanese cedar is known to contain secondary metabolites, such as phenylpropanoid-related compounds in heartwood[11] and tannins in bark,[12] and these metabolites may also have ACE inhibitory activity. In this study, therefore, we screened and isolated ACE inhibitors from 24 extracts prepared from Japanese cedar, including the roots, leaves, heartwood, sapwood, inner bark, and outer bark, by successive extraction with four solvents.

Y. Tsutsumi (✉) · A. Shimada · A. Miyano · T. Nishida
Faculty of Agriculture, Shizuoka University, Shizuoka 422-8529, Japan
Tel. +81-54-238-4853; Fax +81-54-238-4852
e-mail:afytutu@agr.shizuoka.ac.jp

T. Mitsunaga
Faculty of Bioresources, Mie University, Tsu 514-8507, Japan

Materials and methods

Materials and chemicals

Six parts (heartwood, sapwood, inner bark, outer bark, leaves, and roots) of Japanese cedar (tree age 15 years)

were collected in April 1995 in Shizuoka University Forest at Kamiatago, Shizuoka, Japan. All of the chemicals were extra pure grade and were used without further purification. ACE from rabbit lung was a product of Wako, Japan.

Preparation of extracts from Japanese cedar

The 24 extracts were obtained from the meals of six parts (heartwood, sapwood, inner bark, outer bark, leaves, and roots) by successive extraction with four solvents: *n*-hexane, chloroform, ethanol, and hot water. Each extract solution was evaporated under reduced pressure and then stored at −20°C.

Assay of ACE inhibitory activity

The ACE activity in the absence of inhibitor (assay A) or in the presence of inhibitor (assay B) was assayed by fluorometric determination of the amount of histidyl-leucine (His-Leu) released from a substrate, hippuryl-histidyl-leucine (Hip-His-Leu), based on methods described elsewhere.[13,14] An ACE reaction medium of 100 mM HEPES buffer (pH 8.3) containing 300 mM NaCl, 5% dimethylsulfoxide (DMSO), and 5 mM Hip-His-Leu was employed according to the manufacturer's protocol supplied with ACE except for containing 5% DMSO to facilitate the solubility of inhibitors. All the reactions were initiated by the addition of substrate after preincubation of ACE (0.75 mU) at 37°C for 3 min. When ACE activity was determined in the presence of inhibitor, the inhibitor was preincubated for 3 min with the enzyme before adding the substrate. The enzyme reaction was carried out at 37°C for 30 min in a final volume of 1.0 ml and then terminated with 2 ml of 0.1 N NaOH. The His-Leu product in the reaction mixture was estimated following *o*-phthalaldehyde treatment. To the denatured reaction mixture, 0.1 ml of 0.2% *o*-phthalaldehyde in methanol was added to form the *o*-phthalaldehyde condensation product of His-Leu. The mixture was incubated at 0°C for 15 min in the dark; then 0.4 ml of 1.5 M phosphoric acid was added for neutralization. The fluorescence of the *o*-phthalaldehyde condensation product of His-Leu was determined with a spectrophotofluorometer (360 nm excitation, 480 nm emission wavelength). The controls underwent two kinds of fluorometric assay; one was in the absence of both ACE and inhibitor (assay A′), and the other was in the absence only of ACE (the inhibitor was included, assay B′). The ACE activity in the absence of inhibitor (A-A′) was designated 100%. The ACE inhibition (%) was calculated from (A-A′) and (B-B′). To estimate the concentration of each inhibitor giving 50% of ACE inhibition (IC_{50}), at least four varied concentrations of an inhibitor within 10% to 90% of ACE inhibition were employed, and an inhibition (%) vs. inhibitor concentration curve was drawn. All assays including the fluorometric controls were individually repeated eight times; and the averages of six values (excluding the highest and lowest values) were calculated. The IC_{50} value for captopril as a positive control for the ACE inhibitor was determined to be 15.7 nM. The reference values for captopril were 12 nM[9] and 23 nM.[3]

Isolation and identification of active principles

The ethanol extract from outer bark was separated into 11 fractions by high-performance liquid chromatography (HPLC): column, Wakosil-II 5C-18AR (Wako, Japan); solvent, 12.6% methanol in water; flow rate, 1.0 ml/min; detection, 280 nm and refractive index. Further purification of fractions 2 and 6 was performed under the same conditions as above.

Identification of the active principle in fraction 6 was achieved by ^{1}H-NMR (in CD_3OD at 400 MHz) and gas chromatography–mass spectrometry (GC–MS). Fraction 6 was trimethylsilylated with bis(trimethylsilyl)-trifluoroacetamide (BSTFA) in pyridine at 70°C for 30 min and then analyzed by GC–MS (column, OV-1, 0.52 mmϕ × 30 m, temp. 150°–300°C at 5°C/min). Fraction 2 was analyzed by ^{1}H-NMR (in CD_3OD at 500 MHz).

Catechin: ^{1}H-NMR (in CD_3OD): Chemical shift (δ) 2.40 [(1H, dd (double doublet), J (coupling constant) = 16.0, 7.9 Hz)], 2.75 (1H, dd, J = 16.0, 5.5 Hz), 3.87 [(1H, ddd (double double doublet), J = 7.9, 7.7, 5.5 Hz)], 4.47 [(1H, d (doublet), J = 7.7 Hz)], 5.76 (1H, d, J = 2.3 Hz), 5.82 (1H, d, J = 2.3 Hz), 6.61 (1H, dd, J = 8.3, 1.9 Hz), 6.67 (1H, d, J = 8.3 Hz), 6.73 (1H, d, J = 1.9 Hz); GC–MS of pentatrimethylsilyl (TMS) derivative, m/z (mass to charge ratio, %): 650 (M^+, 10.20), 368 (92.69), 355 (25.22), 309 (10.39), 267 (9.72), 249 (2.12), 179 (10.30), 147 (5.77), 73 (100).

Procyanidin B3: ^{1}H-NMR (in CD_3OD): Chemical shift (δ) 2.39 (1H, dd, J = 16.3, 8.1 Hz), 2.66 (1H, dd, J = 16.3, 5.5 Hz), 3.69 (1H, ddd, J = 8.1, 7.3, 5.5 Hz), 4.15 (1H, d, J = 9.8 Hz), 4.25 (1H, dd, J = 9.8, 7.9 Hz), 4.31 (1H, d, J = 7.9 Hz), 4.44 (1H, d, J = 7.3 Hz), 5.68 (1H, d, J = 2.3 Hz), 5.78 (1H, d, J = 2.3 Hz), 5.97 [(1H, s (singlet)], 6.15 (1H, dd, J = 8.2, 2.0 Hz), 6.36 (1H, dd, J = 8.2, 2.0 Hz), 6.49 (1H, d, J = 2.0 Hz), 6.568 (1H, d, J = 8.2 Hz), 6.573 (1H, d, J = 8.2 Hz), 6.64 (1H, d, J = 2.0 Hz).

Results

Screening and isolation of ACE inhibitors from Japanese cedar

The 24 extracts obtained from six parts (heartwood, sapwood, inner bark, outer bark, leaves, and roots) of Japanese cedar by successive extraction with four solvents (*n*-hexane, chloroform, ethanol, and hot water) were subjected to the determination of ACE inhibitory activity (IC_{50}). The results are summarized in Table 1. A wide spectrum of IC_{50} values from 24 extracts show that ethanol extracts have rather high activity (lower IC_{50} value) than the other extracts. The ethanol extract from outer bark showed the highest inhibitory activity (IC_{50} is 16 μg/ml). Thus, isolation of active principles from this extract was attempted by HPLC equipped with a

Table 1. Determination of IC_{50} for each extract prepared from Japanese cedar

Extract	IC_{50} (μg/ml)					
	Leaves	Roots	Outer bark	Inner bark	Sapwood	Heartwood
Hexane	155	190	150	39	+	65
Chloroform	220	+	+	80	75	160
Ethanol	180	25	16	43	85	32
Hot water	+	ND	ND	ND	ND	165

+, Inhibition is less than 50% at 250μg/ml; ND, IC_{50} was not determined because of the low solubility of the sample in the assay system

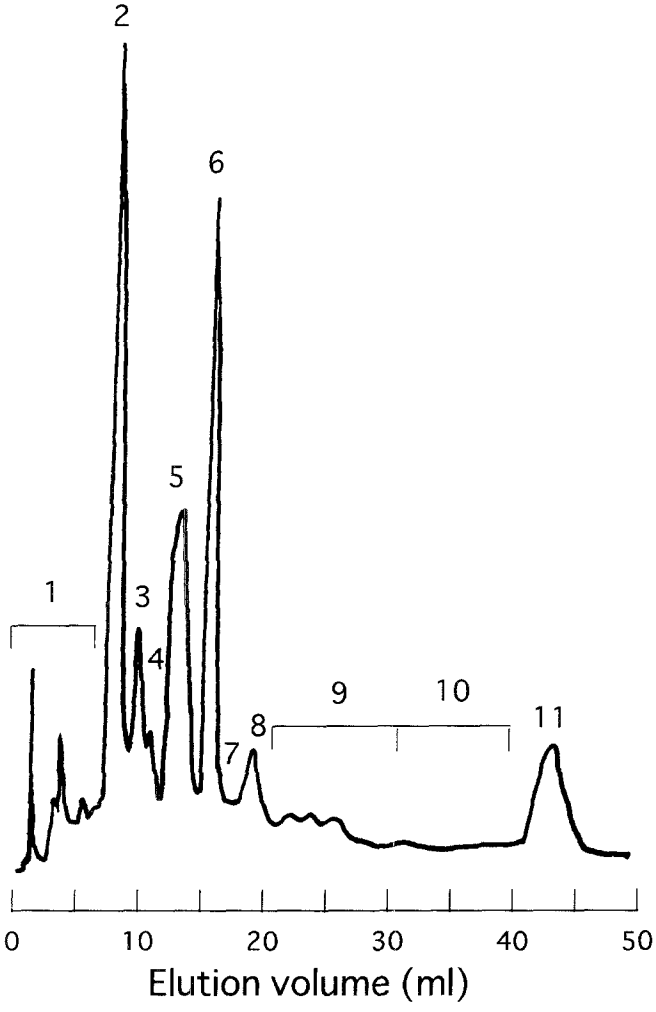


Fig. 1. High-performance liquid chromatography elution profile of the ethanol extract from outer bark. Numbers in the figure correspond to the fraction numbers

Table 2. Determination of IC_{50} for each crude fraction separated from the ethanol extract of Japanese cedar outer bark

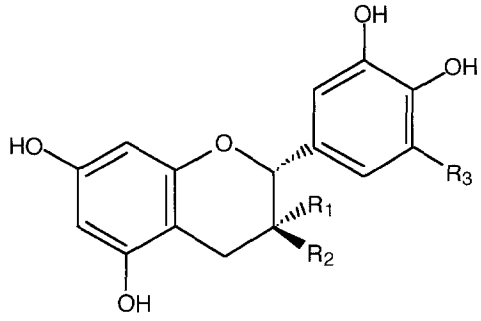
Fraction	IC_{50} (μg/ml)	Yield (%)[a]
1	+	14.9
2	18	8.6
3	25	2.7
4	50	1.9
5	15	7.0
6	23	9.3
7	75	1.6
8	50	2.0
9	35	9.8
10	55	4.1
11	25	6.3
Total		68.2

+, Inhibition is less than 50% at 150μg/ml.
[a] Yield is expressed as percent of the crude ethanol extract

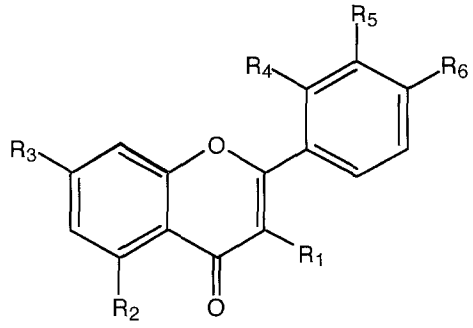
reversed-phase column. The ethanol extract from outer bark was separated into 11 fractions according to the elution profile monitored with ultraviolet absorption at 280nm (Fig. 1). Monitoring with a refractive index detector produced a similar chromatogram. The IC_{50} values and yields of all fractions are listed in Table 2. We found that many fractions possessed ACE inhibitory activity, but most did not show higher activity than the crude ethanol extract from outer bark. Because the total yield of all fractions from the crude ethanol extract was about 70%, it is not likely that we may miss some active principles during the fractionation. It is interesting that the ethanol extract from Japanese cedar outer bark contains several active ACE inhibitors. The partially purified fractions 2 and 6 showed relatively higher ACE inhibitory activity (IC_{50} values are 18 and 23μg/ml, respectively) and higher yield among the separated fractions (Table 2). Therefore, both fractions were further purified by HPLC to identify the active principles. The ^{1}H-NMR spectrum of the purified fraction 6 assigns catechin. The GC–MS spectrum and retention time of the penta-TMS derivative of this compound are identical to those of authentic (+)-catechin. Identification of the other active principle in the purified fraction 2 was performed by ^{1}H-NMR analysis, and the spectrum was assigned as procyanidin B3.

ACE inhibitory activities of flavan-3-ols and flavones

We confirmed that catechin and dimeric procyanidin B3 have high ACE inhibitory activity. The chemical structure required to express ACE inhibitory activity is of interest in these and structurally similar compounds. The commercially available three flavan-3-ols, a flavone, and five fla-



(+)-Catechin:R_1=H, R_2=OH, R_3=H
(−)-Epicatechin:R_1=OH, R_2=H, R_3=H
(−)-Epigallocatechin:R_1=OH, R_2=H, R_3=OH



Flavone:R_1=H, R_2=H, R_3=H, R_4=H, R_5=H, R_6=H
3-Hydroxyflavone:R_1=OH, R_2=H, R_3=H, R_4=H, R_5=H, R_6=H
3,7-Dihydroxyflavone:R_1=OH, R_2=H, R_3=OH, R_4=H, R_5=H, R_6=H
Fisetin:R_1=OH, R_2=H, R_3=OH, R_4=H, R_5=OH, R_6=OH
Quercetin:R_1=OH, R_2=OH, R_3=OH, R_4=H, R_5=OH, R_6=OH
Morin:R_1=OH, R_2=OH, R_3=OH, R_4=OH, R_5=H, R_6=OH

Fig. 2. Chemical structures of the authentic compounds subjected to the angiotensin-converting enzyme inhibitory assay

vonols in Fig. 2 were subjected to the ACE inhibitory activity assay. The IC_{50} values of these compounds are listed in Table 3. Except for flavone and 3-hydroxyflavone, all compounds tested here showed high ACE inhibitory activity (IC_{50} values ranged from 10 to 38 μg/ml). There is no big difference among the IC_{50} values for the three flavonols fisetin, quercetin, and morin, suggesting that the phenolic hydroxyl group at C5 position on A ring is not essential for expressing ACE inhibitory activity. 3,7-Dihydroxyflavone, which has only one phenolic hydroxyl group on the aromatic A ring, showed high inhibitory activity, but flavone and 3-hydroxyflavone did not. Therefore, an aliphatic hydroxyl group at the C3 position may have little effect on the inhibitory activity. The common characteristic of these seven active compounds, including three flavan-3-ols and four flavonols, seems to be a phenolic hydroxyl group at C7 position on aromatic A ring and heterocyclic oxygen. The number or position of the phenolic hydroxyl group(s) on aromatic B ring may be less important for the inhibitory activity than the C7 phenolic hydroxyl group.

Table 3. Determination of IC_{50} for each authentic compound

Compound	IC_{50} (μg/ml)	IC_{50} (μM)
Flavan-3-ols		
(+)-Catechin	16	55.2
(−)-Epicatechin	16	53.4
(−)-Epigallocatechin	38	122.5
Flavone and flavonols		
Flavone	+	+
3-Hydroxyflavone	+	+
3,7-Dihydroxyflavone	10	39.4
Fisetin	22	73.4
Quercetin	27	87.7
Morin	25	81.1

+, Inhibition is less than 50% at 500 μg/ml

Discussion

Although medicinal plant research has revealed hundreds of pharmacologically active principles,[15] the potential of woody plants as sources of drugs is still unexplored because only a small number of the woody plants have been investigated phytochemically. The extracts from woody plants submitted to pharmacological screening are even fewer.[16] Japanese cedar is the most abundant planted woody species, and now new uses for Japanese cedar are expected. To our knowledge, the pharmacological activities of the extracts from Japanese cedar have been investigated only for SOD mimic and tyrosinase inhibitory activities.[1] In this study, we focused on screening ACE inhibitors from Japanese cedar.

During the primary screening of ACE inhibitors from the crude 24 extracts from Japanese cedar, we found that 5 of 24 extracts showed high ACE inhibitory activity (IC_{50} values were less than 50 μg/ml). This is a rather high positive hit rate and high inhibitory activity compared to that in a previous report[4] in which the crude water, ethanol, and acetone extracts from 31 plant species were subjected to in vitro screening of ACE inhibitors; here 12 crude extracts inhibited ACE by more than 50% when 0.33 mg of test compound was employed in 1-ml assay volume. We isolated catechin and procyanidin B3 from Japanese cedar outer bark as active principles. Although the IC_{50} values for procyanidins were not determined, some procyanidins including procyanidin B3 from *Lespedeza capitata*,[6,7] were reported to be ACE inhibitors. However, Wagner and Elbl[6] reported that catechin did not show ACE inhibitory activity. In our study, both partially purified catechin (fraction 6) and procyanidin B3 (fraction 2) were obtained at high yields, 9.3% and 8.6%, from the crude ethanol extract of outer bark, respectively. Our results and the literature suggest that Japanese cedar bark is a promising, economical source of ACE inhibitors.

Furthermore, we showed that two flavan-3-ols [(−)-epicatechin and (−) epigallocatechin)] and four flavonols (3,7-dihydroxyflavone, fisetin, quercetin, and morin) have ACE inhibitory activity. The IC_{50} values for flavan-3-ols and

flavonols tested in this study ranged 39.4 to 122 μM and are comparable to the reported IC_{50} values for tetrahydroxyxanthones.[8] It was reported that ACE inhibitory activities of tetrahydroxyxanthones were lost by blocking their phenolic hydroxyl groups with acetyl groups.[8] Furthermore, tetrahydroxyxanthones with a phenolic hydroxyl group at the C4 or C5 position (e.g., 3,4,6,7-tetrahydroxyxanthone or 1,3,5,6-tetrahydroxyxanthone) show high inhibitory activity (IC_{50} values are 35.4 and 69.2 μM, respectively), but the activities of other tetrahydroxyxanthones (1,3,6,7-tetrahydroxyxanthone and 2,3,6,7-tetrahydroxyxanthone) are lower (IC_{50} values are 530.8 and 769 μM, respectively).[8] Tetrahydroxyxanthones also have a heterocyclic oxygen atom, as do the flavan-3-ols and flavonols. In the case of tetrahydroxyxanthones, the free hydroxyl group at C4 or C5 and the heterocyclic oxygen atom are probably important for chelating the zinc ion to inactivate ACE. It is not likely that two phenolic hydroxyl groups in the *ortho*-position have high inhibitory effect on ACE activity based on the IC_{50} values for tetrahydroxyxanthones. In our study, 3,7-dihydroxyflavone had the highest IC_{50} among the compounds tested. This compound has only one phenolic hydroxyl group on aromatic A ring and none on aromatic B ring, suggesting that the phenolic hydroxyl groups on aromatic B ring may have less effect on ACE inhibitory activity. A comparison of IC_{50} values for fisetin (3,7,3′,4′-tetrahydroxyflavone), quercetin (3,5,7,3′,4′-pentahydroxyflavone), and morin (3,5,7,2′,4′-pentahydroxyflavone) also suggests the lesser importance of the position of phenolic hydroxyl groups on B ring for ACE inhibitory activity. The participation of the phenolic hydroxyl group at C5 position on A ring in inhibitory activity was also considered to be less based on the comparison of four flavonols: 3,7-dihydroxyflavone, fisetin, quercetin, and morin. In our study, all active compounds, flavan-3-ols and flavonols, are characterized by a phenolic hydroxyl group at the C7 position and heterocyclic oxygen. In the literature[7] mesquitol (3-hydroxy-7,8,3′,4′-tetramethoxyflavan), morin, and amentoflavone [5,7,4′-trihydroxyflavone-(3′ → 8)-5,7,4′-trihydroxyflavone] were also reported to be ACE inhibitors, though their IC_{50} values were not determined. Mesquitol is the only ACE inhibitory flavonoid in which the C8 position is substituted with a methoxyl group. According to the results on tetrahydroxyxanthones, flavonoids with a hydroxyl group or a methoxyl group at C8 position may also have high ACE inhibitory activity. However, we do not have enough information about the ACE inhibitory activity of C8-hydroxylated or C8-methoxylated flavonoids, because the occurrence of these types of flavonoid is limited in plants.[17,18] We, therefore, favor the explanation that the phenolic hydroxyl group at the C7 position and heterocyclic oxygen atom in the compounds tested in this study are putative zinc-chelating sites. The hydroxyl group at C3 position was a characteristic feature, as was the C7 phenolic hydroxyl group, with the only exception being that the inhibitory activity of 3-hydroxyflavone was low. The possibility that the C3 hydroxyl group participates in the expression of ACE inhibitory activity cannot be completely ruled out by the only exception, 3-hydroxyflavone. It is more difficult to define the mechanism behind ACE inhibition by dimeric procyanidins including procyanidin B3; more combinations of putative zinc-chelating functional groups should be considered between the two flavan-3-ol units, upper and lower parts. The mechanisms for ACE inhibition by flavonoids and related compounds (e.g., dimeric and oligomeric procyanidins) should be the subject of a forthcoming study.

We demonstrated that catechin and dimeric procyanidin B3 are ACE inhibitors from Japanese cedar outer bark. We also reported that other flavan-3-ols, (−)-epicatechin and (−)-epigallocatechin, and four flavonols have ACE inhibitory activity. These active compounds are generally found as phenylpropanoid-related metabolites in woody plants. The results in this study and literatures suggest that Japanese cedar and other woody plants are good sources of ACE inhibitors.

Acknowledgment This research was supported in part by financial aid from the Scientific Research Fund (no. 08660202) of the Ministry of Education, Science, Sports and Culture of Japan.

References

1. Nishida T, Hanamura Y, Tsutsumi Y (1995) Superoxide dismutase mimic and tyrosinase inhibitory activities of extractives from sugi (*Cryptomeria japonica*). Mokuzai Gakkaishi 41:522–524
2. Ondetti M, Rubin B, Cushman D (1977) Design of specific inhibitors of angiotensin-converting enzyme: a new class of orally active antihypersensitive agents. Science 196:441–447
3. Ondetti MA (1988) Structural relationship of angiotensin converting enzyme inhibitors to pharmacologic activity. Circulation (suppl I) 77:74–78
4. Hansen K, Nyman U, Smitt U, Adsersen A, Gudiksen L, Rajasekharan S, Pushpangadan P (1995) In vitro screening of traditional medicines for anti-hypertensive effect based on inhibition of the angiotensin converting enzyme. J Ethnopharmacol 48(1):43–51
5. Sendl A, Elbl G, Redl K, Breu W, Wagner H (1992) Comparative pharmacological investigations of *Allium ursinum* and *Allium sativum*. Planta Med 58:1–7
6. Wagner H, Elbl G (1992) ACE-inhibitory procyanidins from *Lespedeza capitata*. Planta Med 58:297
7. Wagner H (1993) Leading structure of plant origin for drug development. J Ethnopharmacol 38:105–112
8. Chen C-H, Lin J-Y (1992) Inhibition of angiotensin-I-converting enzyme by tetrahydroxyxanthones isolated from *Tripterospermum lanceolatum*. J Nat Prod 55:691–695
9. Hansen K, Adsersen A, Christensen S, Jensen S, Nyman U, Smitt U (1996) Isolation of an angiotensin converting enzyme (ACE) inhibitor from *Olea europaea* and *Olea lancea*. Phytomedicine 2:319–325
10. Hara Y, Matsuzaki T, Suzuki T (1987) Angiotensin I converting inhibiting activity of tea components (in Japanese). Nippon Nogeikagaku Kaishi 61:803–808
11. Imamura H, Yasue M (1983) List of the extractives from woody plants. In: Imamura H (ed) Chemistry for wood utilization (in Japanese). Kyoritsu Shuppan, Tokyo, pp 324–399
12. Samejima M, Yoshimoto T (1979) Procyanidins from the inner bark of sugi (*Cryptomeria japonica* D. Don). Mokuzai Gakkaishi 25:671–677
13. Cheung HS, Cushman DW (1973) Inhibition of homogeneous angiotensin-converting enzyme of rabbit lung by synthetic venom peptides of *Bothrops jararaca*. Biochim Biophys Acta 293:451–463
14. Tsai B-S, Peach MJ (1977) Angiotensin homologs and analogs as inhibitors of rabbit pulmonary angiotensin-converting enzyme. J Biol Chem 252:4674–4681

15. Hamburger M, Hostettmann K (1991) Bioactivity in plants: the link between phytochemistry and medicine. Phytochemistry (Oxf) 30:3864–3874
16. Tachibana S (1995) Utilization of biologically active substances in trees (in Japanese). Mokuzai Gakkaishi 41:967–977
17. Harborne JB (1989) Flavonoids. In: Powe JW (ed) Natural products of woody plants, vol. I. Springer, pp 533–570
18. Ebel J, Hahlbrock K (1982) Biosynthesis. In: Harborne JB, Mabry TJ (ed) The flavonoids: advances in research. Chapman & Hall, New York, pp 641–679