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Xanthoangelol and 4-Hydroxyderricin Are the Major Active Principles of the Inhibitory Activities against Monoamine Oxidases on *Angelica keiskei* K

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Abstract

Monoamine oxidase inhibitors (MAOI) have been widely used as antidepressants. Recently, there has been renewed interest in MAO inhibitors. The activity-guided fractionation of extracts from *Angelica keiskei* Koidzumi (*A. keiskei* K.) led to the isolation of two prenylated chalcones, xanthoangelol and 4-hydroxyderricin and a flavonoid, cynaroside. These three isolated compounds are the major active ingredients of *A. keiskei* K. to inhibit the MAOs and DBH activities. Xanthoangelol is a nonselective MAO inhibitor, and a potent dopamine β-hydroxylase (DBH) inhibitor. IC_{50} values of xanthoangelol to MAO-A and MAO-B were calculated to be 43.4 μM, and 43.9 μM. These values were very similar to iproniazid, which is a nonselective MAO inhibitor used as a drug against depression. The IC_{50} values of iproniazid were 37 μM, and 42.5 μM in our parallel examination. Moreover, IC_{50} value of xanthoangelol to DBH was calculated 0.52 μM. 4-Hydroxyderricin is a potent selective MAO-B inhibitor and also mildly inhibits DBH activity. The IC_{50} value of 4-hydroxyderricin to MAO-B was calculated to be 3.43 μM and this value was higher than that of deprenyl (0.046 μM) used as a positive control for selective MAO-B inhibitor in our test. Cynaroside is a most potent DBH inhibitor. The IC_{50} value of cynaroside to DBH was calculated at 0.0410 μM. Results of this study suggest that the two prenylated chalcones, xanthoangelol and 4-hydroxyderricin isolated from *A. keiskei* K., are expected for potent candidates for development of combined antidepressant drug. *A. keiskei* K. will be an excellent new bio-functional food material that has the combined antidepressant effect.

Key Words: *Angelica keiskei* Koidzumi, Monoamine oxidase inhibitor, Dopamine β-hydroxylase inhibitor, Xanthoangelol, 4-hydroxyderricin, Cynaroside

INTRODUCTION

Monoamine oxidase (MAO) inhibitors were the first antidepressants introduced, but their use has dwindled because of their reported side effects, their food and drug interactions, and the introduction of other classes of agents. However, there has been renewed interest in MAO inhibitors (Wimbiscus *et al.,* 2010). Recently, Meyer group reported the relationship between MAO-A levels and selective serotonin reuptake inhibitor (SSRI) treatment, recovery, and recurrence in major depressive disorder (MDD). They concluded that from the perspective of monoamine theory, SSRI raise serotonin levels vigorously whereas elevated MAO-A levels would be expected to metabolize serotonin, norepinephrine, and dopamine excessively. The mismatch between monoamine levels raised by treatment and monoamine levels lowered by disease processes might, at times, contribute to lack of response to SSRI treatment (Meyer *et al.,* 2009). Consequently, A MAO-A inhibitor helps on the treatment of depression with a SSRI. On the other hand, Kitaichi group reported that the combined treatment with a MAO-A inhibitor and MAO-B inhibitor strengthens antidepressant effects because the combined treatment increases extracellular norepinephrine levels more than MAO-A inhibitor alone through increases in β-phenylethylamine (Kitaichi *et al.,* 2010). *Angelica keiskei* Koidzumi (Umbelliferae), which is growing mainly along the Pacific coast of Asia is a hardy perennial herb. It has been used traditionally as a diuretic, laxative, analeptic and galactagogue, and has recently gained attention as a health food in Korea. It has been stud-

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ied as folklore medicine for many metabolic diseases, such as hypertension, hepatosis and neuralgia (Kim *et al.,* 1992), and is reported to have numerous biological benefits, such as anti-hyperlipidemic activity (Park *et al.,* 1997), lowering blood pressure (Shimizu *et al.,* 1999), antitumor action (Okuyama *et al.,* 1991), and suppression of gastric acid secretion (Fujita *et al.,* 1992). Various chalcones, coumarins and flavonoids have so far been isolated and characterized from this plant (Baba *et al.,* 1990; Park *et al.,* 1995; Akihisa *et al.,* 2003).

Although Ogawa group reported the dietary 4-hydroxyderricin produces suppression in the elevation of systolic blood pressure (Ogawa *et al.,* 2005), it remains unclear whether *A. keiskei* effects to the nervous system, such as nervous sedative effect, antidepression, and dementia treatment, which could be controlled by MAO or DBH inhibitors.

MAO inhibitors were among the first drugs used in the treatment of depression (Quitkin *et al.,* 1979), Parkinson's disease (PD) (Birkmayer *et al.,* 1983; Riederer *et al.,* 1983; Stern *et al.,* 1983) and schizophrenia (Rigal and Zarifian, 1983). MAO-B inhibitors have been used in the treatment of Parkinson disease. Especially, selective MAO-B inhibitor, selegiline does show success as an adjunctive treatment for Parkinson disease and does not necessitate any dietary restriction. Particularly, DBH inhibitors can provide the added advantage of increasing levels of dopamine (DA) with MAO-B inhibitors.

In the present study, we showed that the two prenylated chalcones, xanthoangelol and 4-hydroxyderricin isolated from *A. keiskei* have inhibitory activities against MAO-A and MAO-B. We also investigated the inhibitory activities of a flavonoid, cynaroside on DBH activities in *in vitro* assay.

MATERIALS AND METHODS

Plant materials

Fresh leaves and stems of *A. keiskei* were collected from Yangju, Korea, and identified by Dr. KW Park, botanist at the Korea National Arboretum. The voucher specimen (NP20-204) has been deposited in the specimen room of Duksung Women's university, Seoul, Korea.

Chemicals and instruments

NMR experiments were performed on a Bruker/Advance-600 (600 MHz). The chemical shifts were reported in parts per million, and the coupling constants (*J* values) were in Hertz. Exact masses were measured by a Hewlett Packard 5890 series II (EI-MS) or Jeol JMSAX505WA Mass spectrometer. Column chromatography was carried out on silica gel 60 (0.063-0.200 μm; Merck 7734) and Lichroprep RP-18 (particle size 40-60 μm, Merck). TLC analyses were carried out on silicagel 60 F254 (Merck 7734) and RP-18 F254s (Merck 15685) plates. In bioassay experiments, UV absorbance was measured by a UVIKON XS UV/Vis spectrometer of SECOMAN. Serotonin creatinine sulfate, Iproniazid, deprenyl Tyramine-HCl, Dowex 50W 8 and Amberlite CG50 were purchased from Sigma Co., USA, and Benzylamine-HCl from Tokyo Kasei Co., Japan.

Animals

Male Sprague-Dawley rats weighing 180-200 *g* were obtained from the Orient Animal Laboratoty (Seoul, Korea) and were maintained on a 12 h light-dark cycle (light phase: 06:30-18:30) in a temperature-controlled environment (22 ± 1°C) with free access to food and water. Experiment began after 10 day period of acclimatization. All procedures were approved by the Kunkuk University Animal Care and Use Committee. They complied with the Guide for the Care and Use of Laboratory Animals, Bio-Food and Drug Research Center Kunkuk University.

Procedures of MAO-A assay *in vitro*

Rat brain mitochondrial MAO was prepared by the method of Kim *et al.* (2012). The activity of MAO-A was measured according to Han *et al.* (2001) using serotonin as the substrate. The reaction mixture containing 0.5 ml of enzyme solution in 10 mM phosphate buffered saline (pH 7.1) and 1 ml of test solution was preincubated at 37°C for 15 min, after which 0.5 ml of 1 mM solution of buffered serotonin creatinine sulfate (Sigma, USA) was added. Following incubation at 37°C for 90 min, the enzyme reaction was terminated by heating the reaction mixture for 3 min in a boiling water bath. After being centrifuged, 1.6 ml of the supernatant, was applied to an Amberlite CG50 column (0.8 i.d.×3 cm). The column was washed with 40 ml of distilled water and eluted with 3 ml of 4N acetic acid. The absorbance of the metabolite that produced after being reacted with MAO-A was measured at 277 nm. Purely isolated compounds were dissolved in DMSO and then suspended in water for testing their inhibitory activities on MAO-A. Final concentration of DMSO in enzymatic reaction mixture was below 5%. Iproniazid was used as positive control for nonselective inhibitor.

Procedures of MAO-B assay *in vitro*

Rat liver mitochondrial MAO was prepared by Zeller's method (Kim *et al.*, 2012). The activity of MAO-B was measured according to Han *et al.* (2001), using benzylamine as the substrate. The reaction mixture containing 0.5 ml of enzyme solution in 10 mM phosphate buffered saline (pH 7.1) and 1 ml of test solution was preincubated at 37°C for 15 min and then cooled in an ice bath. 0.5 ml of 4 mM benzylamine HCl (Tokyo Kasei Co., Japan) in buffer was added to it. This mixture was further incubated at 37°C for 90 min in a shaking water bath. The reaction was terminated by addition of 0.2 ml of 60% perchloric acid. After extraction with 3 ml of cyclohexane, the organic layer was taken and the absorbance of benzaldehyde produced was measured at 242 nm. Purely isolated compounds were dissolved in DMSO and then suspended in water for testing their inhibitory activities on MAO-A. Final concentration of DMSO in enzymatic reaction mixture was below 5%. Deprenyl was used as positive control for selective MAO-B inhibitor.

Procedures of DBH assay *in vitro*

The enzyme activity of DBH was determined according to Kim *et al.* (2012). Using tyramine as the substrate. The following were sequentially added to 0.3 ml of enzyme solution in 0.25 M sucrose: 1 ml of test solution; 0.2 ml of 3 mg/ml catalase; 0.5 ml of 1 M acetate buffer (pH 5.0); and 0.5 ml of a reaction aid, prepared by dissolving fumaric acid, N-ethylmaleimide, iproniazide phosphate and ascorbic acid to concentrations of 0.06, 0.06, 0.006 and 0.06 M, respectively, in distilled water. The solution was allowed to stir at 37°C for 15 min, and then 0.5 ml of 0.12 M tyramine HCl solution was added and the resulting mixture was allowed to stir for 90 min.

Next, 0.4 ml of 3 M solution of trichloroacetic acid was added to the reaction mixture to terminate the enzyme reaction. Immediately thereafter, the solution was centrifuged and 3 ml of the supernatant was poured onto a Dowex 50 W×8 column (0.8 i.d.×3 cm, H+ form, 200-400 mesh) and the column was washed with 30 ml of distilled water. Three milliliters of 4 N NH_4OH solutions were then added to the column. The eluate was collected in a test tube and 0.2 ml of 4% sodium metaperiodate solution was added. The test tube was allowed to stand for 10 min, before 0.2 ml of 20% sodium metabisulfite solution was added. The absorbance of the resulting mixture was measured at 330 nm.

Isolation of xanthoangelol, 4-hydroxyderricin and cynaroside from *A. keiskei*

The dried aerial parts (10 kg) of *A. keiskei* were extracted three times with a mixture of methanol and distilled water (7:3, v/v: 25 L) at 80°C for 4 hr. The combined MeOH extract was concentrated *in vacuo* at 45°C to give 2.8 kg residue. The purification and isolation of two prenylated chalcones and a flavonoid from the EtOAc and n-BuOH extract were performed according to general chromatographic methods. Briefly, the EtOAc and BuOH extract was chromatographed on a silica gel column (Silica gel 60, 70-230 mesh: Merck Japan, Tokyo, Japan) using stepwise gradient elution with the hexane-dihloromethane (1:3), dichloromethane-methanol (30:1), dichloromethane-methanol (3:1) to yield fraction I to VI. The xanthoangelol (3.1 g, 99% purity, yellow needles) was finally purified with a LiChroprep RP-18 Lobar column (Merck, Japan) with 80% methanol from the fraction I (15 g). The 4-hydroxyderricin (400 mg, 98% purity, yellow powders) was finally purified with a LiChroprep RP-18 Lobar column with 80% methanol from the fraction II (6 g). The fraction VI (13 g) was subjected to silica gel column chromatography using dicloromethane-methanol (5:1) to afford cynaroside (300 mg, 99% purity, yellowneedles). These three compounds were identified by direct comparison with an authentic sample (Park *et al*., 1995; Akihisa *et al*., 2003). The structures of the isolated compounds are given in Fig. 1.

Xanthoangelol (1)

Yellowish needles, EI-MS : m/z 392[M]+(C25H28O4), 1H-NMR (600 MHz, CDCl3) : δ 1.59 (3H, s, 7''-CH3), 1.63 (3H, s, 8''-CH3), 1.85 (3H, s, 3''-CH3), 2.08 (2H, m, 4''-H), 2.10 (2H, m, 5''-H), 3.49 (1H, d, J=7.1 Hz, 1''-H), 5.05 (1H, m, 6''-H), 5.30 (1H, t, J=7.1 Hz, 2''-H), 6.43 (1H, d, J=8.9 Hz, 5'-H), 6.88 (2H, d, J=8.5 Hz, 3,5-H), 7.46 (1H, d, J=15.4 Hz, α), 7.55 (2H, d, J=8.5 Hz, 2,6-H), 7.72 (1H, d, J=8.9 Hz, 6'-H), 7.83 (1H, d, J=15.4 Hz, b), 13.88 (1H, s, 2'-OH).

4-Hydroxyderricin (2)

Yellowish powders, EI-MS: m/z 338[M]+(C21H22O4), 1HNMR (600MHz, CDCl3) : δ 1.60 (3H, s, 4''-CH3), 1.71 (3H, s, 5''-CH3), 3.31 (1H, d, J=7.0 Hz,1''-H), 3.82 (3H, s, 4-OCH3), 5.30 (1H, t, J=7.0 Hz, 2''-H), 6.41 (1H, d, J=9.0 Hz, 5'-H), 6.79 (2H, d, J=8.5 Hz, 3,5-H), 7.36 (1H, d, J=15.4 Hz, α), 7.70 (2H, d, J=9.4 Hz, 2,6-H), 7.70 (1H, d, J=9.4 Hz, 6'-H), 7.72 (1H, d, J=15.4 Hz, b), 13.38 (1H, s, 2'-OH).

Cynaroside (3)

Yellowish needles, ^{1}H-NMR (600MHz, DMSO d_6+D_2O) δ 5.04 (1H, d, *J*=7.5 Hz, G1), 6.44 (H, d, *J*=2.0 Hz, 6-H), 6.73 (1H, s, 3-H), 6.80 (1H, d, *J*=2.1 Hz, 8-H), 6.91 (1H, d, *J*=8.4 Hz, 5'-H), 6.91 (1H, d, *J*=8.4 Hz, 5'-H), 7.40 (1H, d, *J*=2.2 Hz, 2'-H), 7.44 (1H, d, *J*=8.4 Hz, 6'-H). ^{13}C-NMR (150MHz, DMSO d_6 + D_2O) : δ 60.8 (G6-C), 69.8 (G4-C), 73.3 (G2-C), 76.4 (G3-C), 77.3 (G5-C), 95.3 (C-8), 99.9 (6-C), 100.2 (G1-C), 105.7 (10-C), 113.7 (2'-C), 116.2 (3-C), 116.3 (5'-C), 119.6 (6'-C), 121.7 (1'-C), 145.9 (3'-C), 150.1 (4'-C), 151.1 (5-C), 157.3 (9-C), 163.2 (7-C), 164.8 (2-C), 182.2 (4-C).

RESULTS

The inhibitory activities of xanthoangelol on MAOs

Each solvent fraction and isolated compounds from Fr. I to VI, concentrated EtOAc and butanol extracts of *A. keiskei* were examined for MAOs inhibitory activities. As shown in Table 1, total MeOH extract and each solvent fraction have inhibitory potential on MAO-A and MAO-B activities. Especially, Dichloromethane and EtOAc fractions showed most potent inhibitory activities against of both enzymes. BuOH fraction also shows the inhibitory activities on both enzymes. EtOAc and BuOH fractions were chosen for elucidating their active principles. Table 1 shows the inhibitory activities of each solvent fraction on MAO-A and MAO-B. The IC_{50} values of the dichloromethane fraction were 0.30 mg/ml for MAO-A, 0.06 mg/ml for MAO-B. The IC_{50} values of the EtOAc and BuOH fractions were 0.09 mg/ml and 1.3 mg/ml for MAO-A, 0.13 mg/ml and

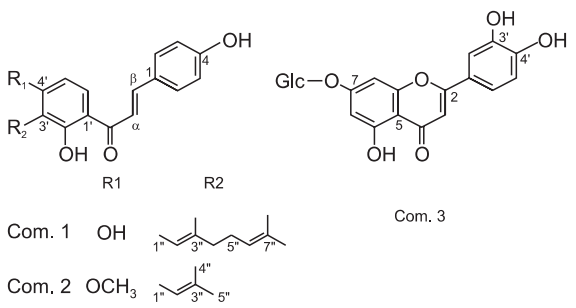

Fig. 1. The Structures of the two prenylated chalcones, xanthoangelol (1) and 4-hydroxyderricin (2) and a flavonoid, cynaroside (3) isolated from *Angelica keiskei.*

Table 1. The IC_{50} values of each solvent extract of *A. keiskei* against MAO-A, MAO-B and DBH activities

Fractions	Amounts of extract (*g*)	IC_{50} values (mg/ml)		
		MAO-A	MAO-B	DBH
MeOH	1,000	0.51	0.33	0.68
Hexane	110	-	1.90	-
CH_2Cl_2	56	0.30	0.06	0.17
EtOAc	45	0.09	0.13	0.08
BuOH	138	1.30	0.85	0.22
Water	400	-	2.75	-

Table 2. The IC_{50} values of the isolated compounds from *A. keiskei* against MAO-A, MAO-B and DBH activities

Compounds	IC_{50} values (micro mole)		
	MAO-A	MAO-B	DBH
Xanthoangelol	43.4	43.9	516
4-hydroxyderricin	3,520	3.43	12.0
Cynaroside	400	268	0.041
Iproniazid[a]	37	42.5	-
Deprenyl[b]	3.3	0.046	-

[a]Used as a positive control drugs for nonselective MAO inhibitor.
[b]Used as a positive control drug for selective MAO-B inhibitor.

0.85 mg/ml for MAO-B, respectively. Table 2 shows the inhibitory activities of the isolated compounds on MAO-A and MAO-B. Xanthoangelol exhibited the inhibitory activities on the both enzymes potentially. The IC_{50} value of xanthoangelol was 43.4 μM for MAO-A, 43.9 μM for MAO-B. In our examinations, the IC_{50} value of the iproniazid, positive control of the nonselective MAO inhibitor was 37 μM for MAO-A and 42.5 μM for MAO-B, respectively. Iproniazid is a nonselective inhibitor of MAOs and deprenyl (selegiline) is a selective MAO-B inhibitor. Iproniazid and deprenyl were used as positive controls for nonselective and selective inhibitors.

The inhibitory activities of xanthoangelol on DBH

As shown in Table 1, total MeOH extract and each solvent fraction have inhibitory potential on DBH activities. Except for the hexane fraction, CH_2Cl_2, EtOAc and BuOH fractions showed lowest IC_{50} values against DBH enzyme. Among them, EtOAc fraction and BuOH fraction were chosen for elucidating their active principles. Table 2 shows the inhibitory activities of the isolated compounds on DBH. Xanthoangelol exhibited the very weak inhibitory activities on DBH. The IC_{50} value of xanthoangelol was 516 μM for DBH.

The inhibitory activities of 4-hydroxyderricin on MAOs

Table 2 shows the inhibitory activities of the isolated compounds on MAO-A and MAO-B. 4-hydroxyderricin exhibited the inhibitory activities on the both enzymes potentially. The IC_{50} value of 4-hydroxyderricin was 3.52 mM for MAO-A, 3.43 μM for MAO-B. In our examinations, the IC_{50} value of the deprenyl, positive control of the selective MAO-B inhibitor was 3.3 μM for MAO-A and 0.046 μM for MAO-B, respectively. 4-Hydroxyderricin was the strongest and selective MAO B inhibitor among the isolated compounds. Its specific activity on MAO-B was about 15 more than that of xanthoangelol, about 150 times more than that of cynaroside, and about 1,000 and 5,000 times more than its own specific activity on MAO-A and DBH. In addition, it exhibited about 1,000 times less IC_{50} value on MAO-B than that on MAO-A, deprenyl showing about 70 times less that on MAO-B than that on MAO-A. This result indicates that 4-hydroxyderricin is a more selective MAO-B inhibitor than deprenyl as a selective MAOB inhibitor.

The inhibitory activities of 4-hydroxyderricin on DBH

As shown in Table 2,4-hydroxyderricin exhibited the inhibitory activities on DBH mildly. The IC_{50} value of 4-hydroxyderricin was 12.0 μM for DBH.

The inhibitory activities of cynaroside on MAOs

Table 2 shows the inhibitory activities of cynariside on MAO-A and MAO-B. Cynaroside was not a good inhibitor for MAO-A and MAO-B. Even though it was very weak, cynaroside exhibited the inhibitory activities on both enzymes. The IC_{50} values of cynaroside were 0.4 mM for MAO-A, 0.27 mM for MAO-B.

The inhibitory activities of cynaroside on DBH

As shown in Table 1, total MeOH extract and each solvent fraction have inhibitory potential on DBH activities. Except the hexane fraction, CH_2Cl_2, EtOAc and BuOH fractions showed potent inhibitory activities against DBH enzyme. Among them, EtOAc fraction and BuOH fraction were chosen for elucidating their active principles. In Table 2, we show the inhibitory activities of the isolated compounds on DBH. Cynaroside exhibited strongest inhibitory activities on DBH among all of the isolated compounds. The IC_{50} value of cynaroside was 0.041 μM for DBH.

DISCUSSION

The activity-guided fractionation of extracts from *Angelica keiskei* Koidzumi led to the isolation of two prenylated chalcones, xanthoangelol and 4-hydroxyderricin and a flavonoid, cynaroside. Three compounds were exhibited the inhibitory activities against MAO-A, MAO-B and DBH respectively. *A. keiskei* is a major vegetable used as a fresh salad. As described in the introduction, traditional use of this plant is not well known, except for some medicinal purposes, such as hypertension, hepatosis and neuralgia (Kim *et al.,* 1992). Reported studies about bioactivities of *A. keiskei* are few. There are some reports such as an anti-hyperlipidemic (Park *et al.,* 1997), lowering blood pressure (Shimizu *et al.,* 1999), antitumor action(Okuyama *et al.,* 1991), and suppression of gastric acid secretion (Fujita *et al.,* 1992). Some chalcones, coumarins and flavonoids have so far been isolated and characterized from this plant (Baba *et al.,* 1990; Park *et al.,* 1995; Akihisa *et al.,* 2003). In this study, we can find out that this plant has also antidepressant effect. Each isolated compound showed different inhibitory pattern.

One of the isolated compounds, xanthoangelol was found to be nonselective MAO inhibitor, because of its inhibitory activities on MAO-A and MAO-B were very similar to iproniazid, nonselective MAO inhibitor. Although its activity was not great, xanthoangelol also inhibited the DBH activity. The other one, 4-hydroxyderricin was a potent selective MAO-B inhibitor. Its IC_{50} value was higher than that of deprenyl, a selective MAO-B inhibitor used as a positive control, but this plant mainly is used as food, this value is significantly low enough. On the other hands, cynaroside showed potent inhibitory activity on DBH. This compound showed very weak inhibitory activity on MAO-A, and exhibited low activity on MAO-B.

Several reports have described the MAO-A inhibition contributes to the mechanism of antidepressant effects of MAO inhibitors more than MAO-B inhibition (Lipper *et al.,* 1979; Mann *et al.,* 1989). Larsen *et al.* (1991) reported that reversible monoamine oxidase inhibitor (RIMA) has equal antidepressant effects to those of irreversible MAO inhibitors. However,

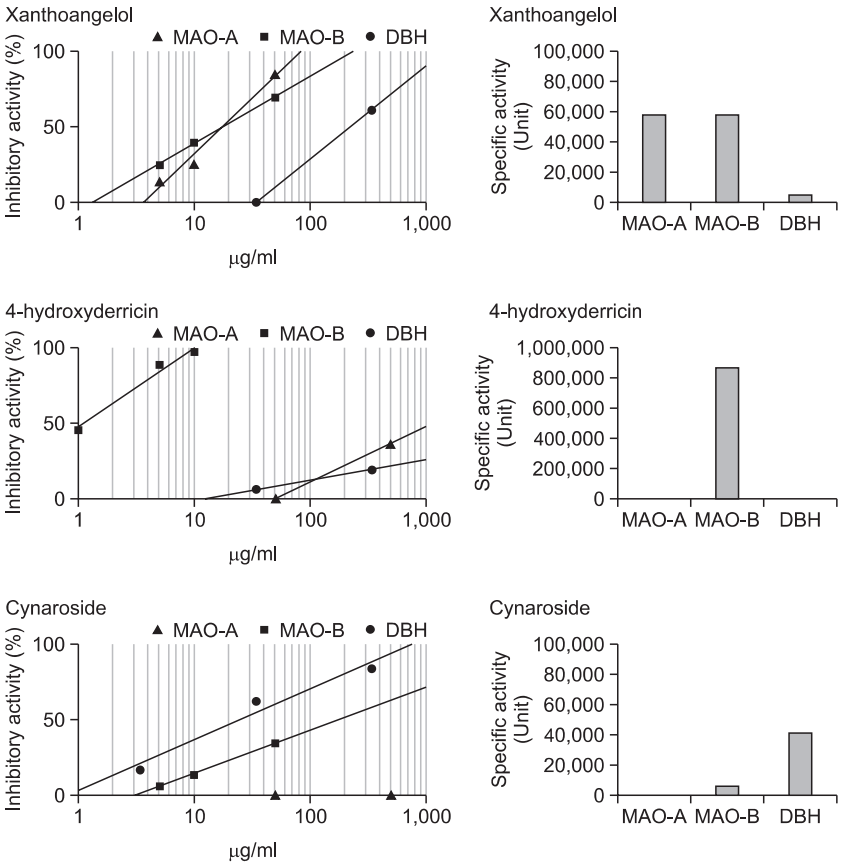

Fig. 2. Inhibitory activities on MAO-A, MAO-B and DBH of the two prenylated chalcones, xanthoangelol (1) and 4-hydroxyderricin (2) and a flavonoid, cynaroside (3) isolated from *Angelica keiskei*. Each compound was tested at concentrations of 1-500 ug/ml) to derive IC_{50} values, and these data obtained mean value from repeated experiments 3 to 5 times of duplicated tests. Specific activity expressed as unit/g, one unit is defined as a sample amount to give 50% inhibition against enzyme activity.

Lotufo-Neto *et al.* (1999) examined antidepressant effects of MAO inhibitors in a meta-analysis and described the possibility that non-selective MAO inhibitors are more effective than RIMA. Consequently, it is likely that MAO-B inhibition also contributes to an antidepressant effects. Kitaichi *et al.* (2010) measured extracellular noradrenaline and serotonin levels after administration of RIMA and reversible MAO-B inhibitor in the medial prefrontal cortec of rats using the *in vivo* micro dialysis method. And they suggested that the combined treatment with a MAO-A inhibitor and a MAO-B inhibitor strengthens antidepressant effects because the combined treatment increases extracellular noradrenaline levels more than a MAO-A inhibitor alone through increases in β-phenylethylamine. According to the results of this study, *A. keiskei* is an excellent combined MAO inhibitor for treatment for depression, because its major bioactive compounds, xanthoangelol, 4-hydroxyderricin and cynaroside were selective, nonselective MAO inhibitor and DBH inhibitor, respectively. Xanthoangelol is a nonselective MAO inhibitor, 4-hydroxyderricin is a selective MAO-B inhibitor, and cynaroside is a selective DBH inhibitor.

There are more MAO-B than MAO-A in the human brain, but more MAO-A than MAO-B in the rat brain (Riederer *et al.,* 1983). The distribution of MAO-A and MAO-B is different between the human brain and the rat brain. The role of MAO-B in antidepressant effects might be greater in human than in rat; stronger antidepressant effects of combined treatment with a MAO-A inhibitor and a MAO-B inhibitor might be likely to be induced in humans (Kitaichi *et al.,* 2010). Results of this study suggest that xanthoangelol, as a nonselective MAO inhibitor; can be potentially used as a drug candidates against depression. 4-Hydroxyderricin, as a selective MAO-B inhibitor, can be potentially used as a drug candidate for this kind of disease. Cynaroside, as a selective DBH inhibitor, can effectively elevate the level of released dopamine (DA) by preventing DBH from converting DA to norepinephrine and being destroyed by oxidative deamination effect of MAO. Thus, that seems to be useful materials for antidepressant drug for human.

As shown in Fig. 2, the degree and the way of inhibition against each enzyme of the isolated compound was different. Xanthoangelol was nonselective between MAO-A and MAO-B. Its inhibitory potentials (IC_{50} value, total activity, and specific activity) on MAO-A were no less than those on MAO-B and about 10 times more than those on DBH. In addition, Its IC_{50} values on MAOs were at a rate comparable with those of iproniazid used as positive control. 4-Hydroxyderricin was the strongest selective MAO B inhibitor among the isolated compounds. Its specific activity on MAO-B was about 15 times more than that of xanthoangelol, about 150 times more than that of cynaroside, and about 1,000 and 5,000 times more than its own specific activity on MAO-A and DBH. In addi-

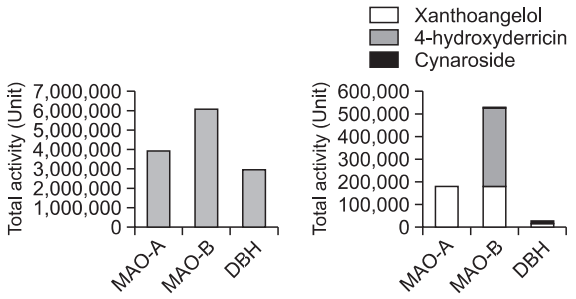

Fig. 3. Total activities of each compound on MAO-A, MAO-B and DBH. The left graph is showing the total activity of the MeOH extract of *A. keiskei* and the right one, a cumulative graph, showing cumulative total activity of the isolated compounds. Each compound was tested at concentrations of 1-500 μg/ml) to derive IC_{50} values, and these data obtained mean value from repeated experiments 3 to 5 times of duplicated tests. Total activity expressed as unit, one unit is defined as a sample amount to give 50% inhibition against enzyme activity.

tion, it exhibited about 1,000 times less IC_{50} value on MAO-B than that on MAO-A, deprenyl showing about 70 times less that on MAO-B than that on MAO-A. As Fig. 3 shows, against MAOs and DBH, total activities of the methanol extract (the left graph) were about 10 to 100 times more than accumulated total activities of the isolated compounds (the right one). However, the both graphs showed similar tendency of the total activities. The activities on MAO-B were more than those on the other enzymes and the activities on DBH were lowest. These results strongly suppose that the isolated compounds are the major active components of *Angelica keiskei* against MAOs and DBH. This result indicates that 4-hydroxyderricin is more selective MAO-B inhibitor than deprenyl used as selective MAO-B inhibitor. Cynaroside was a selective DBH inhibitor. It exhibited inhibitory activity against DBH at concentrations below 0.9 μM, but did not on MAO-A and MAO-B.

In conclusion, two prenylated chalcones, xanthoangelol and 4-hydroxyderricin and a flavonoid, cynaroside isolated from *A. keiskei* were major active principles of this plant. The possibility exists that these three compounds isolated from *A. keiskei* are expected for potent candidates for development of combined antidepressant drug, and the plant *A. keiskei* contained xanthoangelol, 4-hydroxyderricin and cynaroside, as major active components, and will be an excellent functional food material for combined antidepressant effect.

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