Structure-activity relationships of five myricetin galloylglycosides from leaves of *Acacia confusa*

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ABSTRACT. Five structure-related myricetin galloylglycosides isolated from leaves of *Acacia confusa* were previously reported (Lee et al., 2000, J. Nat. Prod., 63, 710-712). However, the structure-activity relationships were not reported. In this research, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, and inhibitory activities against semicarbazide-sensitive amine oxidase (SSAO) and angiotensin converting enzyme (ACE) were compared among five compounds, namely, myricetin 3-*O*-(3"-*O*-galloyl)-α-rhamnopyranoside 7-methyl ether (**compound 1,** 630 Da), myricetin 3-*O*-(2"-*O*-galloyl)-α-rhamnopyranoside 7-methyl ether (**compound 2,** 630 Da), myricetin 3-*O*-(2"-*O*-galloyl)-α-rhamnopyranoside (**compound 3,** 616 Da), myricetin 3-*O*-(3"-*O*-galloyl)-α-rhamnopyranoside (**compound 4,** 616 Da), myricetin 3-*O*-(2", 3" -di-*O*-galloyl)-α-rhamnopyranoside (**compound 5,** 768 Da). For DPPH scavenging activity, the IC_{50} for five compounds was 591, 1522, 3210, 1389, and 867 μM, respectively. For SSAO inhibitory activity, the IC_{50} for five compounds was 36.16, 93.20, 119.50, 88.20, and 39.35 μM, respectively. The IC_{50} of positive control of semicarbazide was 34.21 μM. The five compounds have the same orders of compound 1> compound 5> compound 4> compound 2> compound 3 for DPPH scavenging activity and SSAO inhibitiny. It was found that gallic acid in the R_3 position was the key role for both biological activities. For ACE inhibitory activity, compound 1, compound 2, and compound 5 showed dose-dependent inhibitory modes and the IC_{50} was 60.32, 151.90, and 19.82 μM, respectively.

Keywords: *Acacia confusa*; Angiotensin converting enzyme (ACE); 1, 1-diphenyl-2-picrylhydrazyl (DPPH); Myricetin galloylglycoside; Semicarbazide-sensitive amine oxidase (SSAO); Structure-activity relationships (SAR).

INTRODUCTION

Free radical-mediated reactions are involved in degenerative or pathological processes such as aging (Harman, 1995), cancer, coronary heart disease, and Alzheimer's disease (Ames, 1983; Smith et al., 1996; Diaz et al., 1997). Meanwhile, there are many epidemiological results revealing an association between a diet rich in fresh fruit and vegetable and a decrease in the risk of cardiovascular diseases and certain forms of cancer in humans (Salah et al., 1995). Several reports concern natural compounds in fruits and vegetables for their antioxidant activities, such as phenolic compounds (Rice-Evans et al., 1997), anthocyanin (Espin et al., 2000), echinacoside in *Echinaceae* root (Hu and Kitts, 2000), methanolic and hot-water extracts of *Liriope spicata* L. (Hou et al., 2004), the storage proteins of sweet potato root (Hou et al., 2001a), yam tuber (Hou et al., 2001b), potato tuber (Liu et al., 2003b), and yam mucilages (Hou et al., 2002; Lin et al., 2005).

The semicarbazide-sensitive amine oxidase (SSAO, EC 1.4.3.6), which contains a cofactor of one or more topaquinone, is a common name for a group of heterogenous enzymes widely distributed in nature, in plants, microorganisms, and the organs of mammals (vasculature, dental pulp, eye and plasma) (Boomsma et al., 2000). SSAO converts primary amines into the corresponding aldehydes, generating hydrogen peroxide and ammonia. It was found that the endogenous compounds aminoacetone and methylamine are good substrates for most SSAOs (Precious et al., 1988). In recent research, plasma SSAO was found raised in diabetes mellitus and heart failure and implicated in roles in atherosclerosis, endothelial damage, and glucose transport into adipocytes (Yu and Zuo, 1993, 1996; Boomsma et al., 1997, 2003).

Several classes of pharmacological agents have been used in the treatment of hypertension (Mark and Davis, 2000). One class of anti-hypertensive drugs known as angiotensin I converting enzyme (ACE) inhibitors (i.e. peptidase inhibitors) has a low incidence of adverse side-effects and is the preferred class of anti-hypertensive agents when treating patients with concurrent secondary

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diseases (Fotherby and Panayiotou, 1999). ACE (EC 3.4.15.1) is a dipeptide-liberating exopeptidase which has been classically associated with the renin-angiotensin system regulating peripheral blood pressure (Mullally et al., 1996). The potent ACE inhibitors were frequently derived from food proteins (Ariyoshi, 1993; Hsu et al., 2002). However, pomegranate juice (Aviram and Dornfeld, 2001), flavan-3-ols and procyanidins (Actis-Goretta et al., 2003), and tannins (Liu et al., 2003a) were also reported to have ACE inhibitory activity.

Acacia confusa (Leguminosae) is widely distributed on the hills and lowlands of Taiwan. Five structure-related myricetin galloylglycosides (Figure 1)—namely, myricetin 3-*O*-(3"-*O*-galloyl)-α-rhamnopyranoside 7-methyl ether (**compound 1,** 630 Da), myricetin 3-*O*-(2"-*O*-galloyl)-α-rhamnopyranoside 7-methyl ether (**compound 2,** 630 Da), myricetin 3-*O*-(2"-*O*-galloyl)-α-rhamnopyranoside (**compound 3,** 616 Da), myricetin 3-*O*-(3"-*O*-galloyl)-α-rhamnopyranoside (**compound 4,** 616 Da), and myricetin 3-*O*-(2", 3"-di-*O*-galloyl)-α-rhamnopyranoside (**compound 5,** 768 Da)—isolated from leaves of *Acacia confusa* were previously reported (Lee et al., 2000). However, the structure-activity relationships (SAR) were not reported. In this research, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, and inhibitory activities against SSAO and ACE were compared among five compounds.

MATERIALS AND METHODS

Materials

ACE (I unit, rabbit lung) was purchased from Fluka Chemie GmbH (Switzerland); DPPH, benzylamine, 2', 2-azinodi(3-ethylbenzthiazoline-6-sulfonic acid, ABTS), bovine plasma (P-4639, reconstituted with 10 ml deionized water), horseradish peroxidase (148 units/mg solid), N-(3-[2-furyl] acryloyl)-Phe-Gly-Gly (FAPGG), semicarbazide, and other chemicals and reagents were from Sigma Chemical Co. (St. Louis, MO, USA).

Extraction and purificaction of five structure-related myricetin galloylglycosides from *Acacia confusa*

Five structure-related flavonol galloylglycosides isolated from leaves of *Acacia confusa* were previously reported (Lee et al., 2000). The methanolic extracts were isolated in series by Sephadex LH-20 column, Si-flash column, and reverse-phase HPLC. Each structure was identified by ^{13}C and ^{1}H NMR, COSY, HMQC and HMBC spectra. The purity of each compound was higher than 99%, determined by reverse-phase HPLC.

Scavenging activity against DPPH radical analyzed by spectrophotometry

The scavenging activity of five structure-related flavonol galloylglycosides against DPPH radical was measured according to the method of Hou et al. (2001a, b). Each sample was dissolved in DMSO to a final concentration of 2 mg/ml as stock solutions. Each 0.3 ml sample solution (compound 1, 158.73, 317.46, 476.19, 634.92 μM; compound 2, 317.46, 555.56, 793.65, 1587.3 μM; compound 3, 811.69, 1623.38, 2435.06, 3246.75 μM; compound 4, 487.01, 811.69, 1217.53, 1623.38 μM; compound 5, 390.63, 520.83, 651.04, 976.56 μM) was added to 0.1 ml of 1 M Tris-HCl buffer (pH 7.9) and then mixed with 0.6 ml of 100 μM DPPH in methanol for 20 min under light protection at room temperature. The decrease of absorbance at 517 nm was measured and expressed as $\Delta A517$. Means of triplicates were measured. DMSO was used as a blank experiment. The scavenging activity of DPPH radicals (%) was calculated with the equation: $(\Delta A517_{blank} - \Delta A517_{sample}) \div \Delta A517_{blank} \times 100\%$. The IC_{50} stands for the concentration of 50% scavenging activity.

SSAO inhibitory activities of five structure-related myricetin galloylglycosides

SSAO inhibitory activity was determined by spectrophotometric method according to Szutowicz et al. (1984) with some modifications. The total 200 μl reaction solution [containing 50 μl of 200 mM phosphate buffer, pH 7.4, 50 μl of 8 mM benzylamine, bovine plasma (containing SSAO, 2.53 units) and different amounts of sample solution (compound 1, 15.87, 31.75, 47.62, 63.49 μM; compound 2, 47.62, 63.49, 95.24, 111.11 μM; compound 3, 81.17, 113.64, 129.87, 194.81 μM; compound 4, 64.94, 97.40, 129.87, 162.34 μM; compound 5, 26.04, 39.06, 52.08, 65.10 μM) and semicarbazide (6.25, 12.5, 25, and 50 μM)] was placed at 37°C for one h and then heated at 100°C to stop reaction. After cooling and a brief centrifugation, the 90 μl reaction solution was isolated and added to the 710 μl solution containing 200 μl of 200 mM phosphate buffer (pH 7.4), 100 μl of 2 mM ABTS solution, and 25 μl of horseradish peroxidase (10 μg/ml). The changes of absorbance at 420 nm were recorded during 1 min and expressed as $\Delta A420$ nm/min. Means of triplicates were measured. DMSO was used as a blank experiment. The SSAO inhibition (%) was calculated with the equation: $(\Delta A420$ nm/min$_{blank} - \Delta A420$ nm/min$_{sample}) \div \Delta A420$ nm/min$_{blank} \times 100\%$. The IC_{50} stands for the concentration of 50% inhibitions.

Determination of ACE inhibitory activity of structure-related myricetin galloylglycosides by spectrophotometry.

The ACE inhibitory activity was measured according to the method of Holmquist et al. (1979) and Lee et al. (2003) with some modifications. The 15 μl (15 mU) commercial ACE (1 U/mL, rabbit lung) were mixed with 50 μl of sample solution (compound 1, 14.76, 44.60, 79.21, 148.90 μM; compound 2, 44.60, 74.44, 148.90, 178.73 μM; compound 5, 12.11, 24.35, 36.59, 48.83 μM) and then 1 mL of 0.5 mM FAPGG [dissolved in 50 mM

Tris-HCl buffer (pH 7.5) containing 0.3 M NaCl] was added. The decreased absorbance at 345 nm ($\Delta A_{inhibitor}$) was recorded during 5 min at room temperature. DMSO was used instead of sample solution for blank experiments (ΔA_{blank}). The ACE activity was expressed as $\Delta A345$ nm, and the ACE inhibition (%) was calculated as follows: $[1-(\Delta A_{inhibitor} \div \Delta A_{control})] \times 100\%$. Means of triplicates were determined.

RESULTS AND DISCUSSION

Flavonoids are one type of polyphenol—a group which includes isoflavones, flavones, and flavanones—and have been reported to exhibit many biological activities (Hodnick et al., 1994; Hoult et al., 1994; Siess et al., 1995; Naasani et al., 1998; Lee et al., 2001). Lee et al. (2000) isolated five structure-related myricetin galloylglycosides (Figure 1), and the IC_{50} of anti-hatch activity against brine shrimp from four of them was 50 µg/ml (79.37 µM), 89 µg/ml (141.27 µM), 75 µg/ml (121.75 µM), and 64 µg/ml (83.33 µM), respectively, for compound 1, compound 2, compound 4, and compound 5. Compound 3 was not measured. The four compounds ranked for their anti-hatch activity against brine shrimp take the order: compound 1> compound 5> compound 4> compound 2. Chang et al. (2001) reported the antioxidant activity of 70% ethanolic extracts from *Acacia confusa* bark and heartwood. In this research, the DPPH radical scavenging activity and inhibitory activities against SSAO and ACE were compared among five myricetin galloylglycosides.
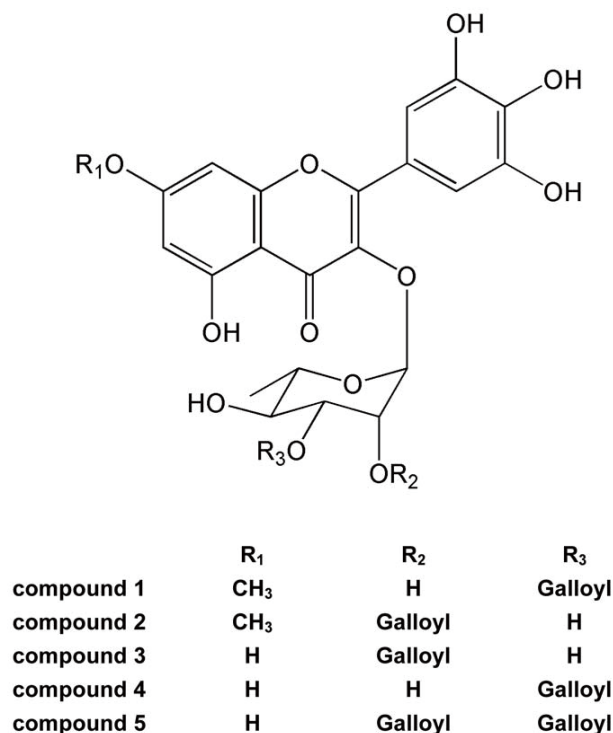

	R_1	R_2	R_3
compound 1	CH_3	H	Galloyl
compound 2	CH_3	Galloyl	H
compound 3	H	Galloyl	H
compound 4	H	H	Galloyl
compound 5	H	Galloyl	Galloyl

Figure 1. Structures of five myricetin galloyoglycosides isolated from leaves of *Acacia confusa*.
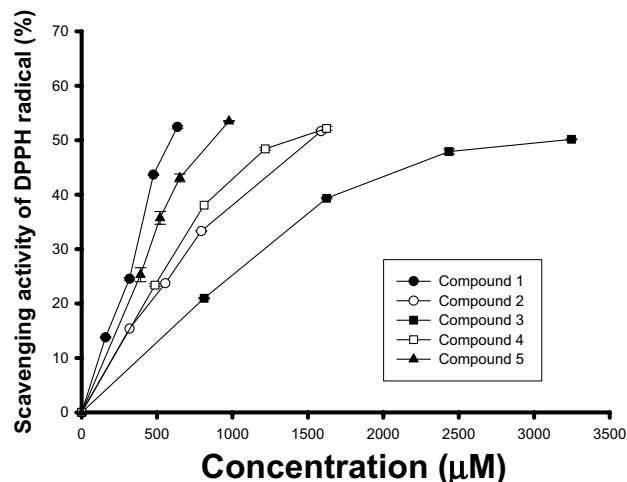

Figure 2. The scavenging activity of five myricetin galloylglycosides against DPPH radicals. Means of triplicates were measured. The scavenging activity of DPPH radical (%) was calculated according to the following equation: ($A517_{blank} - A517_{sample}$) $\div A517_{blank} \times 100\%$.

Scavenging activitiy against DPPH radical analyzed by spectrophotometry

DPPH radicals were widely used in the model system to investigate the scavenging activities of several natural compounds. When DPPH radical was scavenged, the color of the reaction mixture changed from purple to yellow with decreasing of absorbance at wavelength 517 nm. Figure 2 shows the scavenging activity against DPPH radicals from five structure-related myricetin galloylglycosides. The dose-dependent DPPH radical scavenging activities from five pure compounds were found. For compound 1, the scavenging activities against DPPH were 13.86, 24.71, 43.74, and 52.59%, respectively, for 158.73, 317.46, 476.19, 634.92 µM; for compound 2, the scavenging activities against DPPH were 15.36, 23.71, 33.39, and 51.86%, respectively, for 317.46, 555.56, 793.65, 1587.3 µM; for compound 3, the scavenging activities against DPPH were 20.87, 39.40, 47.58, and 50.25%, respectively, for 811.69, 1623.38, 2435.06, 3246.75 µM; for compound 4, the scavenging activities against DPPH were 23.21, 38.06, 48.25, and 52.42%, respectively, for 487.01, 811.69, 1217.53, 1623.38 µM; for compound 5, the scavenging activities against DPPH were 24.37, 36.56, 42.57, and 53.42%, respectively, for 390.63, 520.83, 651.04, 976.56 µM. The IC_{50} of each compound against DPPH radial was shown at Table 1. The IC_{50} for five compounds were 591, 1522, 3210, 1389, and 867 µM, respectively. The five compounds had the order of compound 1> compound 5> compound 4> compound 2> compound 3 for DPPH scavenging activity, which was the same order as for anti-hatch activity against brine shrimp (Lee et al., 2000). For anti-DPPH activity, with the same methyl group in the R_1 position, the gallic acid in the R_3 position (compound 1) was more effective (2.5 fold) than in the R_2 position (compound 2), and this efficiency was

Table 1. Comparisons of the DPPH scavenging activity and inhibitory activities against SSAO and ACE among five myricetin galloyoglycosides isolated from leaves of *Acacia confusa*.

Myricetin galloylglycoside	Scavenging activity of DPPH radical $(\mu M)^a$	SSAO inhibitory activity $(\mu M)^a$	ACE inhibitory activity $(\mu M)^a$
Compound 1	591	36.16	60.32
Compound 2	1522	93.20	151.90
Compound 3	3210	119.50	ND[b]
Compound 4	1389	88.20	ND
Compound 5	867	39.35	19.82

[a]Expressed as the IC_{50} value.
[b]Not detectable.

also found in compound 3 and compound 4. With the same hydrogen atom in the R_1 position, the gallic acid in the R_3 position (compound 4) was more effective (2.3 fold) than in the R_2 position (compound 3). With the same gallic acid in the R_3 position and hydrogen atom in the R_2 position, the methyl group in the R_1 position (compound 1) was more effective (2.35 fold) than the hydrogen atom in the same position (compound 4), and this efficiency was also found in compound 2 and compound 3. With the same gallic acid in the R_2 position and the hydrogen atom in the R_3 position, the methyl group in the R_1 position (compound 2) was more effective (2.11 fold) than the hydrogen atom in the same position (compound 3). While, with two gallic acid groups in the R_2 and R_3 positions (compound 5) were more effective than one in the R_2 position (compound 3) or in the R_3 position (compound 4). It was concluded that the gallic acid in the R_3 position was the key for anti-DPPH activity among five structure-related compounds.

SSAO inhibitory activities of five structure-related myricetin galloylglycosides

Figure 3 shows the SSAO inhibitory activity from five structure-related myricetin galloylglycosides. The dose-dependent SSAO inhibitory activities from five pure compounds were found. For compound 1, the SSAO inhibitory activities were 12.17, 41.44, 67.05, and 81.44%, respectively, for 15.87, 31.75, 47.62, 63.49 μM; for compound 2, the SSAO inhibitory activities were 20.83, 33.47, 50.65, and 74.43 %, respectively, for 47.62, 63.49, 95.24, 111.11 μM; for compound 3, the SSAO inhibitory activities were 25.36, 44.55, 60.85, and 72.58 %, respectively, for 81.17, 113.64, 129.87, 194.81 μM; for compound 4, the SSAO inhibitory activities were 33.45, 56.72, 71.64, and 88.44%, respectively, for 64.94, 97.40, 129.87, 162.34 μM; for compound 5, the SSAO inhibitory activities were 31.99, 48.93, 76.48, and 88.44 %, respectively, for 26.04, 39.06, 52.08, 65.10 μM. The IC_{50} of SSAO inhibitory activity of each compound was shown at Table 1. For SSAO inhibitory activity, the IC_{50} for five compounds was 36.16, 93.20, 119.50, 88.20, and 39.35 μM, respectively. The IC_{50} of semicarbazide (positive control) was 34.21 μM which was close to that
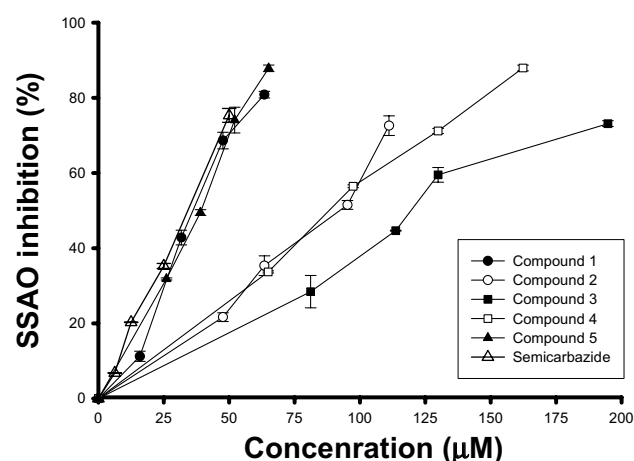

Figure 3. The effects of five myricetin galloylglycosides on the activities of semicarbazide-sensitive amine oxidase (SSAO, 2.53 units) from bovine plasma. The semicarbazide (6.25, 12.5, 25, and 50 μM) was used as a positive control. Deionized water was used as a blank experiment. The changes of absorbance at 420 nm were recorded during 1 min and expressed as $\Delta A420$ nm/min. The SSAO inhibition (%) was calculated with the equation: $(\Delta A420 \text{ nm/min}_{blank} - \Delta A420 \text{ nm/min}_{sample}) \div \Delta A420 \text{ nm/min}_{blank} \times 100\%$.

of compound 1. The five compounds had the order of compound 1> compound 5> compound 4> compound 2> compound 3, which was the same as for DPPH scavenging activities (above-mentioned) and anti-hatch activity against brine shrimp (Lee et al., 2000). For SSAO inhibitory activity, with the same methyl group in the R_1 position, the gallic acid in the R_3 position (compound 1) was more effective (2.6 fold) than in the R_2 position (compound 2), and this efficiency was also found in compound 3 and compound 4. With the same hydrogen atom in the R_1 position, the gallic acid in the R_3 position (compound 4) was more effective (1.35 fold) than in the R_2 position (compound 3). With the same gallic acid in the R_3 position and the hydrogen atom in the R_2 position, the methyl group in the R_1 position (compound 1) was more effective (2.44 fold) than the hydrogen atom in the same position (compound 4), and this efficiency was also found in compound 2 and compound 3. With the same gallic acid

in the R_2 position and hydrogen atom in the R_3 position, the methyl group in the R_1 position (compound 2) was more effective (1.28 fold) than the hydrogen atom in the same position (compound 3). Two gallic acid groups in the R_2 and R_3 positions (compound 5) were more effective than one in the R_2 position (compound 3) or in the R_3 position (compound 4). It was concluded that the gallic acid in the R_3 position was the key role for SSAO inhibitory activity among five structure-related compounds.

ACE inhibitory activity of myricetin galloylglycosides by spectrophotometry

Figure 4 shows the ACE inhibitory activity from five structure-related myricetin galloylglycosides. It was found that three out of five compounds had dose-dependent ACE inhibitory activities. For compound 1, the ACE inhibitory activities were 34.33, 46.27, 54.48, and 64.90 %, respectively, for 14.76, 44.60, 79.21, 148.90 μM; for compound 2, the ACE inhibitory activities were 35.80, 47.02, 49.25, and 56.71 %, respectively, for 44.60, 74.44, 148.90, 178.73 μM; for compound 5, the ACE inhibitory activities were 37.31, 57.46, 67.91, and 71.64 %, respectively, for 12.11, 24.35, 36.59, 48.83 μM. The IC_{50} of ACE inhibitory activity of each compound was shown at Table 1. The IC_{50} of compound 1, compound 2, and compound 5 was 60.32, 151.90, and 19.82 μM, respectively. For ACE inhibitory activity, with the same methyl group in the R_1 position, the gallic acid in the R_3 position (compound 1) was more effective (2.52 folds) than in the R_2 position (compound 2). Two gallic acid groups in the R_2 and R_3 positions (compound 5) were more effective than one in the R_2 position (7.66 folds, compound 2) or in the R_3 position (3.04 folds, compound 1). Liu et al. (2003) reported that gallotannins of five gallic acid groups of 1,2,3,4,6-penta-O-galloyl-β-D-glucose (IC_{50}, 73.1 μM) was more effective in ACE inhibitory activity than four gallic acid groups of 1,2,3,6-tetra-O-galloyl-β-D-glucose (IC_{50}, 101.4 μM). The IC_{50} of two or three gallic acid groups in gallotannin were higher than 200 μM.

In conclusion, the SAR of DPPH radical scavenging activity and inhibitory activities of SSAO and ACE in five structure-related myricetin galloylglycosides were reported. The five compounds have the same orders of compound 1> compound 5> compound 4> compound 2> compound 3 for DPPH scavenging activity and SSAO inhibition. It was postulated that gallic acid in the R_3 position was the key role for anti-DPPH radicals and SSAO inhibitory activity. For ACE inhibitory activity, the orders were compound 5> compound 1> compound 2. It was postulated that more gallic acid groups were the key to ACE inhibitory activity.

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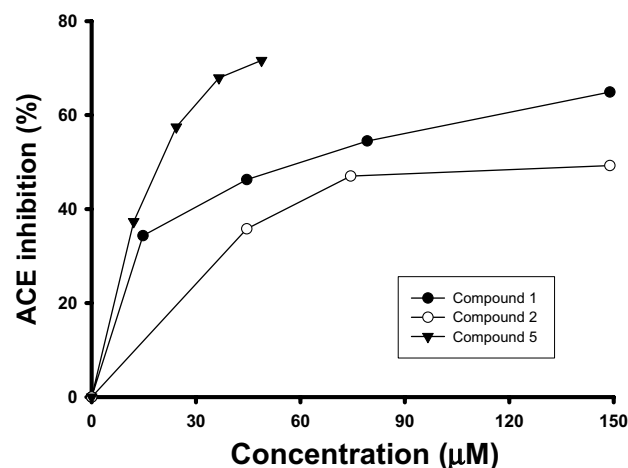Diaz, M.N., B. Frei, J.A. Vita, and J.F. Keaney. 1997.

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相思樹葉子五種楊梅樹皮素配糖體之結構－活性關係

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　　從相思樹葉子分離五種楊梅樹皮素配糖體在之前就已經有報告 (Lee et al., 2000, J. Nat. Prod., 63, 710-712)，但是結構-活性之間的關係並沒有報告。本篇研究將比較五種分離的化合物，包括 myricetin 3-*O*-(3”-*O*-galloyl)-α-rhamnopyranoside 7-methyl ether (化合物 **1**，分子量 630 Da), myricetin 3-*O*-(2”-*O*-galloyl)-α-rhamnopyranoside 7-methyl ether (化合物 **2**，分子量 630 Da), myricetin 3-*O*-(2”-*O*-galloyl)-α-rhamnopyranoside (化合物 **3**，分子量 616 Da), myricetin 3-*O*-(3”-*O*-galloyl)-α-rhamnopyranoside (化合物 **4**，分子量 616 Da), 及myricetin 3-*O*-(2”, 3”-di-*O*-galloyl)-α-rhamnopyranoside (化合物 **5**，分子量 768 Da)，對於清除 DPPH 自由基與抑制 Semicarbazide-sensitive 胺酶（SSAO）及血管收縮素轉化酶（ACE）活性的關係。就清除 DPPH 自由基方面，五種化合物 50% 抑制所需濃度分別為 591, 1522, 3210, 1389, 及 867 μM。就抑制 Semicarbazide-sensitive 胺酶方面，五種化合物 50% 抑制所需濃度分別為 36.16, 93.20, 119.50, 88.20, 及 39.35 μM。就上面清除 DPPH 自由基與抑制 Semicarbazide-sensitive 胺酶的結果可以發現，五種結構相關化合物顯現出以下相同次序：化合物 1> 化合物 5> 化合物 4> 化合物 2> 化合物 3，而 gallic acid 位在 R3 的位置，與上述兩種活性有密切的關係。就抑制血管收縮素轉化酶（ACE）方面，只有化合物 1，化合物 2，及化合物 5 具有濃度相關抑制活性, 三種化合物 50% 抑制所需濃度分別為 60.32, 151.90, 及 19.82 μM。

關鍵詞：相思樹；DPPH 自由基；Semicarbazide-sensitive 胺酶；血管收縮素轉化酶；楊梅樹皮素配糖體；結構-活性關係。