J. Korean Wood Sci. Technol. 2017, 45(4): 456~462

pISSN: 1017-0715 eISSN: 2233-7180
https://doi.org/10.5658/WOOD.2017.45.4.456

Original Article

Phenolic Compounds from Japanese anise (*Illicium anisatum* L.) Twigs[1]

Hee-Jeong Min[2] · Young-Soo Bae[2,†]

ABSTRACT

Japanese anise (*Illicium anisatum* L.) twigs were collected and ground after drying, then immersed with 50% aqueous acetone for 3 days. After filtration, the extracts were fractionated with *n*-hexane, chloroform ($CHCl_3$), ethylacetate (EtOAc) and H_2O, and then freeze-dried after condensation. A portion of EtOAc soluble fraction (5.7 g) was chromatographed on a Sephadex LH-20 column with various aqueous MeOH-H_2O. Compound **2** and compound **3** were isolated from fraction 8 and 5, respectively. Compound **1** and compound **4** were isolated after rechromatography of fraction 7. The isolated compounds were elucidated as (+)-catechin (**1**), taxifolin (**2**), taxifolin-3-*O*-*β*-D-(+)-xylopyranose (**3**) and quercitrin (**4**) by spectral and literature data, and by comparison with the authentic samples. Of the isolated compounds, taxifolin (**2**), taxifolin-3-*O*-*β*-D-(+)-D-xylopyranose (**3**) and quercitrin (**4**) were isolated, for the first time, from the extracts of japanese anise twigs.

Keywords : Japanese anise tree (*llicium anisatum* L.), twigs extracts, phenolic compounds, column chromatography

1. INTRODUCTION

The World Health Organization (WHO) estimates that 80% of the people in developing countries rely on the traditional medicines for their primary health care needs and that 85% of the medicines are from the extracts of plants (Farnsworth *et al*., 1985). Plants constitute a rich source of bioactive chemicals and there are largely free from adverse effects and have excellent pharmacological actions (Kador *et al*., 1985a; 1985b). Therefore, recently there have been many studies to evaluate biological activities of various natural resources (Ko, 2011; Yang, 2011; Ko and Choi, 2015).

Over 70 of *Illicium* species are distributed in China and most of them are indigenous to Southwestern and Eastern China. Chinese star anise are included in the Pharmacopoeia of the Peoples's Republic of China.

Illicium anisatum L., the toxic plant belonging to *Illiciacease*, is distributed throughout eastern Asia where it is used as an ornamental plant and is also found in Jeju Island and the

---

[1] Date Received May 30, 2017, Date Accepted July 10, 2017

[2] Department of Forest Biomaterials Engineering, College of Forest and Environmental Sciences, Kangwon National University, Chuncheon 24341, Republic of Korea

[†] Corresponding author: Young-Soo Bae (e-mail: bae@kangwon.ac.kr)

southern part of Korean peninsula. It is well known as a toxic plant and contains sesquiterpene, anisatin, shikimic acid, which is responsible for its toxicity (Yamada *et al*., 1968).

Recent domestic studies have shown that japanese anise tree possess the inhibitory activities of aldose reductase, lipase, glycation, anti-elastase and anti-inflammatory as well as antioxidant activity (Kim and Oh, 1999; Kim and Kim, 2003; Kim and Kang, 2005; Kim *et al*., 2009). However, there are little studies on the chemical constituents of the tree in domestic because it has toxic although some studies had already done about the biological effects and the analysis of GC-MS on volatile components (Shibuya *et al*., 1978).

Therefore, we investigated the chemical constituents of the extracts of japanese anise twigs for future functional use in this work, and elucidated the structures of isolated phenolic compounds.

2. MATERIALS and METHODS

2.1. Plant material

Fresh Japanese anise twigs were collected at Seogwipo, Jeju-do in January 2017, air dried for two weeks and then ground to fine particles to be extracted. The origin of this plant was comfirmed by Kim Chan-Soo, manager of Warm and Subtropical Forest Research Center, National Institute of Forest Service.

2.2. Sample preparation

The ground twigs (0.66 kg) were immersed in 50% aqueous acetone at room temperature for 3 days. After three times extraction and filtration, the filtrates were combined together and evaporated on a rotary evaporator under the reduced pressure at 40℃. The aqueous crude residue was successively fractionated on a separatory funnel and freeze-dried to give n-hexane (0.8234 g), CH_2Cl_2 (0.5673 g), EtOAc (5.8 g), and H_2O (28.9 g) soluble fractions.

2.3. Structure analysis

^1H and ^{13}C-NMR spectra were recorded on a Bruker (USA) Avance DPX 400 (400 MHz for ^1H and 100 MHz for ^{13}C) and 700 (700 MHz for ^1H and 175 MHz for ^{13}C) spectrometers using TMS (Tetramethylsilane) as an internal standard and chemical shift was given in δ (ppm).

MALDI-TOF-MS were performed with a Micromass Autospec M363 spectrometer.

Thin layer chromatography (TLC) was done on DC-Plastikfolien Cellulose F (Merck) plates and developed with TBAW (t-BuOH-HOAc-H_2O (3 : 1 : 1, v/v/v)) and 6% aqueous HOAc. The spot was detected by illuminating ultraviolet light (UV, 254 and 365 nm) and by spraying vanillin (Vanillin-EtOH-H_2SO_4 (15 : 250 : 2.5, w/v/v)), then heating.

2.4. Column chromatography

A portion of EtOAc fraction (5.7 g) was chromatographed on a Sephadex LH-20 column, successively eluting with MeOH-H_2O (1 : 9 → 3 : 7 → 5 : 5 → 7 : 3 → 9 : 1, v/v) to divide 13 fractions.

Fraction 7 was rechromatographed with MeOH-H_2O (4 : 1, v/v) to yield compound **1** (0.2007 g) and **4** (0.2543 g). Compound **2** (1.0178 g) and **3** (0.1139 g) were isolated from fraction 8 and 5, respectively.

2.4.1. Compound 1

Yellowish amorphous powder, R_f: 0.53 (TBAW) and 0.41 (6% HOAc).

MALDI-TOF-MS : Calculated for $C_{15}H_{14}O_6$ 290, Found *m/z* 313 $[M+Na]^+$, 291 $[M+H]^+$

^{1}H-NMR (CD_3OD, δ) : 2.51 (1H, *dd*, *J* = 8.2 and 16.1 Hz, H-4), 2.85 (1H, *dd*, *J* = 5.4 and 16.1 Hz, H-4), 3.98 (1H, *m*, H-3), 4.57 (1H, *d*, *J* = 8.2 Hz, H-2), 5.86 (1H, *d*, *J* = 2.3 Hz, H-6), 5.93 (1H, *d*, *J* = 1.9 Hz, H-8), 6.75 (1H, *dd*, *J* = 1.9 and 8.1 Hz, H-6), 6.76 (1H, *d*, *J* = 8.1 Hz, H-5), 6.97 (1H, *d*, *J* = 1.9 Hz, H-2).

^{13}C-NMR (CD_3OD, ppm) : 28.55 (C-4), 68.84 (C-3), 82.88 (C-2), 95.53 (C-8), 96.32 (C-6), 100.85 (C-10), 115.28 (C-2'), 116.12 (C-5'), 120.08 (C-6'), 132.24 (C-1'), 146.26 (C-3'), 146.28 (C-4'), 157.86 (C-7), 157.61 (C-5), 156.95 (C-9).

2.4.2. Compound 2

Brownish amorphous powder, R_f: 0.76 (TBAW) and 0.38 (6% HOAc).

MALDI-TOF-MS : Calculated for $C_{15}H_{12}O_7$ 304, Found *m/z* 327 $[M+Na]^+$, 305 $[M+H]^+$

^{1}H-NMR (CD_3OD, δ) : 4.51 (1H, *d*, *J* = 11.49 Hz, H-3), 4.91 (1H, *d*, *J* = 11.51 Hz, H-2), 5.88 (1H, *d*, *J* = 2.04 Hz, H-6), 5.92 (1H, *d*, *J* = 2.11 Hz, H-8), 6.80 (1H, *d*, *J* = 8.09 Hz, H-5'), 6.85 (1H, *dd*, *J* = 1.97 and 8.15 Hz, H-6'), 6.97 (1H, *d*, *J* = 1.95 Hz, H-2').

^{13}C-NMR (CD_3OD, ppm) : 73.87 (C-3), 85.32 (C-2), 96.61 (C-8), 97.63 (C-6), 102.12 (C-10), 116.20 (C-2'), 116.47 (C-5'), 121.30 (C-6'), 130.08 (C-1'), 145.49 (C-3'), 147.34 (C-4'), 164.72 (C-9), 165.44 (C-5), 168.88 (C-7), 198.69 (C-4).

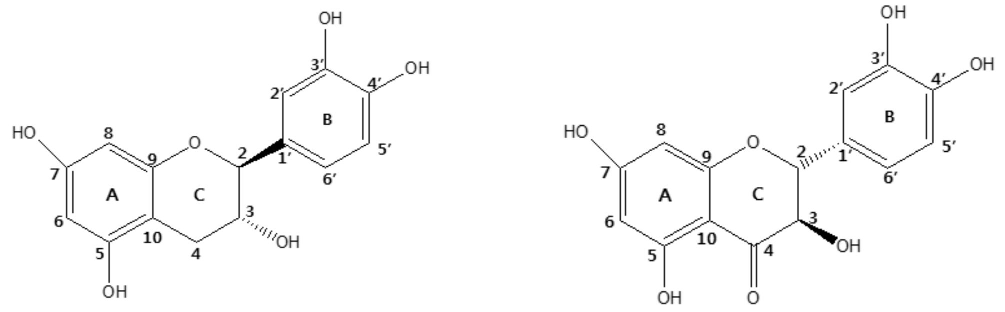
2.4.3. Compound 3

Yellowish amorphous powder, R_f: 0.69 (TBAW) and 0.64 (6% HOAc).

MALDI-TOF-MS : Calculated for $C_{21}H_{22}O_{12}$ 466, Found *m/z* 489 $[M+Na]^+$, 467 $[M+H]^+$

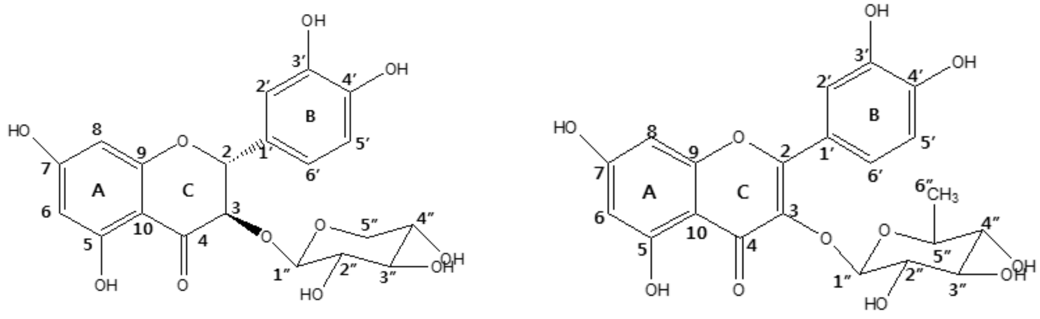
^{1}H-NMR (CD_3OD, δ) : 3.06 (1H, *m*, H-5"b), 3.23 (2H, *m*, H-2", 4"), 3.49 (1H, *m*, H-3"), 3.87 (1H, *d*, *J* = 6.27 Hz, H-1"), 3.94 (1H, *dd*, *J* = 4.65 and 11.77 Hz, H-5"a), 4.74 (1H, *d*, *J* = 9.91 Hz, H-3), 5.20 (1H, *d*, *J* = 9.81 Hz, H-2), 5.90 (1H, *s*, H-6), 5.91 (1H, *s*, H-8), 6.80 (2H, *m*, H-5', 6'), 6.85 (1H, *dd*, *J* = 1.97 and 8.15 Hz, H-6'), 6.95 (1H, *d*, *J* = 1.89 Hz, H-2').

^{13}C-NMR (CD_3OD, ppm) : 65.95 (C-5"), 70.81 (C-3"), 73.43 (C-2"), 75.79 (C-4"), 77.47 (C-3), 83.61 (C-2), 96.37 (C-8), 97.37 (C-6), 102.51 (C-1"), 103.07 (C-10), 115.69 (C-2'), 116.27 (C-5'), 120.87 (C-6'), 128.96 (C-1'), 146.50 (C-3'), 147.35 (C-4'), 164.11 (C-9), 165.48 (C-5), 168.96 (C-7), 195.63 (C-4).



(+)-catechin (**1**)

Taxifolin (**2**)



Taxifolin-3-*O*-*β*-D-(+)-xylopyranose (**3**)

Quercitrin (**4**)

Fig. 1. Chemical structures of isolated compounds.

2.4.4. Compound 4

Yellowish amorphous powder, R$_f$: 0.58 (TBAW) and 0.25 (6% HOAc).

MALDI-TOF-MS : Calculated for $C_{21}H_{20}O_{11}$ 448, Found m/z 471 [M+Na]$^+$, 449 [M+H]$^+$

^{1}H-NMR (CD_3OD, δ) : 0.95 (3H, *d*, *J* = 6.14 Hz, H-6”), 3.42 (1H, *m*, H-5”), 3.66 (1H, *m*, H-4”), 3.76 (1H, *dd*, *J* = 3.40 and 3.23 Hz, H-3”), 4.23 (1H, *dd*, *J* = 1.63 and 1.66 Hz, H-2”), 5.36 (1H, *d*, *J* = 1.5 Hz, H-1”), 6.20 (1H, *d*, *J* = 2.0 Hz, H-6), 6.36 (1H, *d*, *J* = 2.0 Hz, H-8), 6.91 (1H, *d*, *J* = 8.5 Hz, H-5’), 7.31 (1H, *dd*, *J* = 2.2 and *J* = 8.5 Hz, H-6’), 7.34 (1H, *d*, *J* = 2.2 Hz, H-2’).

^{13}C-NMR (CD_3OD, ppm) : 17.69 (C-6”), 71.93 (C-5”), 72.06 (C-3”), 72.13 (C-2”), 73.28 (C-4”), 94.74 (C-8), 99.83 (C-6), 103.56 (C-1”), 105.92 (C-10), 116.39 (C-2’), 116.96 (C-5’), 122.92 (C-6’), 122.99 (C-1’), 136.26 (C-3), 146.42 (C-3’), 149.80 (C-4’), 158.52 (C-9), 159.32 (C-2), 163.21 (C-5), 165.87 (C-7), 179.65 (C-4).

3. RESULTS and DISCUSSION

The compounds were isolated from the EtOAc fraction of the extracts of japanese anise (*Illicium ansisatum* L) twigs by column chromatography using Sephadex LH-20, and the structures were characterized by NMR analysis and by comparison with the other literature data.

3.1. Compound **1**

Compound **1** was obtained as a yellowish amorphous powder from the spring of japanese anise tree. The ^{1}H-NMR spectrum showed the presence of two meta-coupled doublet protons at δ 5.86 (J = 2.3 Hz) and 5.93 (J = 1.9 Hz) in the A-ring, which were assigned to H-6 and H-8, respectively and ascribed to phloroglucinol A-ring. Two sets of doublets and one double doublet at δ 6.97 (J = 1.9 Hz), 6.75 (J = 1.9 and 8.1 Hz) and 6.76 (J = 8.1 Hz) derived from H-2', H-6', and H-5', respectively, suggesting the presence of catechol B-ring. In addition, a doublet at δ 4.57 induced by H-2, a multiplet at δ 3.98 from H-3, and two double doublets at δ 2.51 and 2.85 from H-4 indicated a flavan skeleton.

The ^{13}C-NMR spectrum gave a methylene carbon at δ 28.55 (C-4) and two oxygenated methine carbons at δ 68.84 (C-3) and 82.88 (C-2), which are characteristic of a heterocyclic C-ring (Agrawal, 1989). On the basis of the above spectral evidence, compound **1** was identified as (+)-catechin (Foo *et al*., 1983).

3.2. Compound **2**

Compound **2** was isolated as a brownish amorphous powder from the spring of japanese anise tree. The ^{1}H-NMR spectrum indicated two meta-coupled doublets at δ 5.88 and δ 5.92 revealed the existence of A-ring. The signal at δ 6.80, δ 6.85 and δ 6.97 exhibited characteristic proton peak for B-ring. The flavanol skeleton of compound **2** was easily discerned from the ^{1}H-NMR spectrum by the characteristic set of doublets at δ 4.51 for H-3 and δ 4.91 for H-2.

In the ^{13}C-NMR spectum, the signals at 85.32 ppm, 73.87 ppm and 198.69 ppm were assigned to C-2, C-3 and C-4, respectively, supporting the flavanonol skeleton (Agrawal, 1989). The rest of the ^{13}C-NMR spectral data ascribed to phloroglucinol A-ring and catechol B-ring.

Consequently, compound **2** was elucidated as taxifolin, which was good agreement with the literature (Harbone and Mabry, 1982; Ishimaru *et al*., 1995).

3.3. Compound **3**

Compound **3** was isolated as a yellowish amorphous powder from the spring of japanese anise twigs. ^{1}H-NMR and the ^{13}C-NMR spectra of the aglycon were similar to those of compound **2** which was elucidated as taxifolin.

In the ^{1}H-NMR spectrum of the sugar moiety, two protons of H-5 gave at δ 3.06 and δ 3.94. H-2 and H-4 showed multiplet signals at δ 3.23. H-3 indicated a multiplet signal at δ 3.49 and anomeric H-1 gave a typical doublet signal at δ 3.87 with β coupling constant of 6.27 Hz.

In the ^{13}C-NMR spectum of the sugar moiety, the oxygen-containing C-1 was resonated at 102.51 ppm due to the conjugation with C-3 of taxifolin. The other carbons, C-2, C-3, C-4 and C-5, gave typical signals of xylose at 73.43 ppm, 70.81 ppm, 75.79 ppm and 65.95 ppm, respectively (Agrawal, 1989).

Accordingly, compound **3** was elucidated as taxifolin-3-*O*-β-D-(+)-xylopyranose, which was also coincided by comparison of the literature data (Dubeler *et al.*, 1996; Bergeron *et al.*, 1997; Agrawal, 1989).

3.4. Compound **4**

Compound **4** was obtained as a yellowish amorphous powder from the twigs of japanese anise.

The ^{1}H-NMR spectrum of aglycon gave three proton signals at δ 7.34, δ 7.31, and δ 6.91 attributable to H-2', H-6', and H-5', respectively. Also two meta-coupled doublets at δ 6.20 and δ 6.36 identical to H-6 and H-8, respectively. In the sugar moiety, H-2 indicated a double doublet signal at δ 4.23. The signal at δ 3.76, δ 3.66 and δ 3.42 showed H-3, H-4 and H-5, respectively. An anomeric proton signal at δ 5.36 with α coupling constant of 1.5 Hz and a methyl proton signal at δ 0.95 were observed, which are characteristic of α-L-rhamnopyranose.

In the ^{13}C-NMR spectrum of the sugar moiety, C-1 and C-6 were observed at 103.56 ppm and 17.69 ppm, respectively, which are characteristic of α-L-rhamnopyranose. The other carbons, C-2, C-3, C-4 and C-5, gave 72.13 ppm, 72.06 ppm, 73.28 ppm and 71.93 ppm, respectively. And the rest ^{13}C-NMR spectral data ascribed to phloroglucinol A-ring and catechol B-ring of quercetin.

On the basis of the above spectral data and by comparison of the literature data (pyo *et al.*, 2002; Lee *et al.*, 2004), compound **4** was elucidated as quercetin-3-*O*-α-L-rhamnopyranose, quercitrin.

4. CONCLUSION

Japanese anise (*Illicium anisatum* L) twigs were collected, air-dried and extracted with 50% aqueous acetone. The extracts were concentrated and then sequentially fractionated with n-hexane, CH_2Cl_2, EtOAc, and H_2O to be freeze-dried. A portion of EtOAc fraction (5.7 g) was chromatographed on a Sephadex LH-20 column, by the successively elution with various aqueous MeOH-H_2O (1 : 9 → 3 : 7 → 5 : 5 → 7 : 3 → 9 : 1, v/v).

Compound **2** and **3** were isolated from fraction 8 and 5, respectively. Fraction 7 was rechromatographed with MeOH-H_2O (4 : 1, v/v) to yield compound **1** and **4**.

The isolated compounds were elucidated as (+)-catechin (**1**), taxifolin (**2**), taxifolin-3-*O*-β-D-(+)-xylopyranose (**3**) and quercitrin (**4**) by the spectral and literature data, and by comparison with the authentic samples. Of the isolated compounds, taxifolin (**2**), taxifolin-3-*O*-β-D-(+)-xylopyranose (**3**) and quercitrin (**4**) were isolated, for the first time, from the extracts of japanese anise twigs and these compounds can be used valuable index markers for the tree species.

ACKNOWLEDGEMENTS

This study was supported by the Basic Research Program for Forest Science of Korean Forest Service (No S211315L010130) and also partially supported by Kangwon National University.

REFERENCES

Agrawal, P.K. 1989. Carbon-13 NMR of Flavonoids. Elsevier, Amsterdam.

Bergeron, C., Marston, A., Antus, S., Gauthier, R., Hostettmann, K. 1998. Flavonoids from Pyrola elliptica. Phytochemistry 49(1): 233～236.

Dubeler, A., Volmer, G., Gora, J., Lunderstadt, J., Zeeck, A. 1997. Phenols from Fagus sylvatica and their role in defence against Cryptococcus fagisuga. Phytochemistry 45(1): 51～57.

Farnsworth, N.R., Akerele, O., Bingel, A.S. 1985. Medicinal plants in therapy. Bulletin of the World Health Organization 63(6): 965～981.

Foo, L.Y., McGraw, G.W., Hemingway, R.W. 1983. Condensed tannins: Preferential substitution at the interflavanoid bond by sulphite ion. Journal of the Chemical Society, Chemical Communications 12: 672～673.

Harborne, J.B., Mabry, T.J. 1982. The flavonoid : advances in research, Chapman and Hall Ltd.

Isimaru, K., Omoto, T., Asai, K.E., Shimonura, K. 1995. Taxifolin-3-arabinoside from Fragaria X Ananassa. Phytochemistry 40(1): 345～347.

Kador, P.F., Robison, W.G., Kinoshita, J.H. 1985a. The pharmacology of aldose reductase inhibitors. Annual Review Pharmacology, Toxicology 25: 691～714.

Kador, P.J., Konishita, J.H., Sharpless, N.E. 1985b. Aldose reductase inhibitors: a potential new class of agents for the pharmacological control of certain diabetic complications. Journal of Medicinal Chemistry 28: 841～849.

Kim, H.Y., Oh, J.H. 1999. Screening of Korean forest plants for rat lens aldose reductase inhibition. Bioscience, Biotechnology and Biochemistry 63: 184～188.

Kim, H.Y., Kim, K. 2003. Protein glycation inhibitory and antioxidative activities of some plant extracts in vitro. Journal of Agricultural and Food Chemeistry 51: 1586～1591.

Kim, H.Y., Kang, M.H. 2005. Screening of Korean medicinal plants for lipase inhibitory activity. Phytotherapy Research 19: 359～361.

Ko, E.K., Choi, S.E. 2015. Inhibitory effects of phenolic compounds from stems of *Acer ginnala* on nitric oxide production. Journal of Chemical and Pharmaceutical Research 7(2): 395～402

Ko, R.K., Kim, G.O., Hyun, C.G., Jung, D.S., Lee, N.H. 2011. Compounds with tyrosinase inhibition, elastase inhibition and DPPH radical scavenging activities from the branches of *Distylium racemosum* Sieb. et Zucc. Phytotherapy research 25: 1451～1456.

Lee, J.H., Chung, H.K., Baek, N.I., Kim, S.H., Hee, W.P., Dae, K.K. 2004. Phytochemical constituents from Diodia teres. Archives of Pharmacal Research 27(1): 40～43.

Pyo, M.K., Koo, Y.K., Yun-Choi, H.S. 2002. Anti-platelet effect of the phenolic constituents isolated from the leaves of Magnolia obovata. Natural Product Sciences 8(4): 147～151.

Shibuya, M., Abe, K., Nakahahsi, Y., Kubota S. 1978. Phenolic components from leaf oil of *Illicium anisatum* L. Chemical and Pharmaceutical Bulletin 26(9): 2671～2673.

Yang, Z.G., Jia, L.N., Shen, Y., Ohmura, A., Kitanaka, S. 2011. Inhibitory effects of constituents from *Euphorbia lunulata* on differentiation of 3T3-L1 cells and nitric oxide production in RAW264.7 Cells. Molecules 16: 8305～8318.

Yamada, K., Takada, S., Nakamura, S., Hirata, Y. 1968. Structures of anisatin and neoanisatin toxic sesquiterpenes from Illicium anisatum. Tetrahedron 24(1): 199～229.