Taylor & Francis
Taylor & Francis Group

Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

Anti-inflammatory isocoumarins from the bark of *Fraxinus chinensis* subsp. *rhynchophylla*

Beom Zoo Lee, Chan-Sik Kim, Soon-Kyu Jeong, Sulhae Lee, Ik Soo Lee & KwangWon Hong

To cite this article: Beom Zoo Lee, Chan-Sik Kim, Soon-Kyu Jeong, Sulhae Lee, Ik Soo Lee & KwangWon Hong (2020): Anti-inflammatory isocoumarins from the bark of *Fraxinus chinensis* subsp. *rhynchophylla*, Natural Product Research, DOI: 10.1080/14786419.2020.1715401

To link to this article: https://doi.org/10.1080/14786419.2020.1715401

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2020.1715401

Taylor & Francis
Taylor & Francis Group

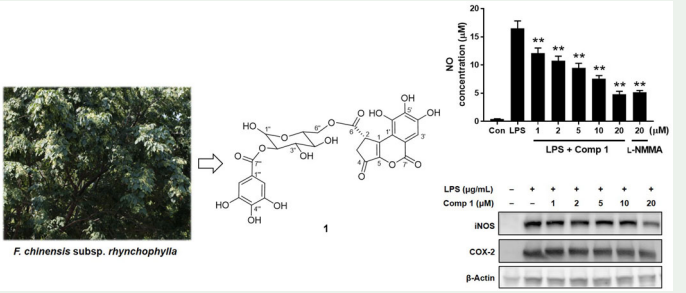
Anti-inflammatory isocoumarins from the bark of *Fraxinus chinensis* subsp. *rhynchophylla*

Beom Zoo Lee[a,b], Chan-Sik Kim[c], Soon-Kyu Jeong[b], Sulhae Lee[b], Ik Soo Lee[c] and KwangWon Hong[a]

[a]Department of Food Science and Biotechnology, College of Life Science and Biotechnology, Dongguk University, Goyang, Republic of Korea; [b]Chemland Co., Ltd., Gunpo, Republic of Korea; [c]Herbal Medicine Research Division, Korea Institute of Oriental Medicine, Daejeon, Republic of Korea

ABSTRACT

A new isocoumarin (**1**) named fraxicoumarin was isolated from the bark of *Fraxinus chinensis* subsp. *rhynchophylla* along with three known compounds (**2**–**4**). The structure of the new compound was established by extensive spectroscopic studies and chemical evidence. The anti-inflammatory effects of the isolated compounds (**1**–**4**) on lipopolysaccharide (LPS)-induced RAW 264.7 macrophage cells were evaluated *in vitro*. Of the compounds tested, compounds **1** and **3** inhibited LPS-induced nitric oxide (NO) production in RAW 264.7 cells. Consistent with these findings, they also suppressed LPS-induced expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) at the protein level in RAW 264.7 cells.



ARTICLE HISTORY
Received 18 October 2019
Accepted 4 January 2020

KEYWORDS
Fraxinus chinensis subsp. *rhynchophylla*; isocoumarins; anti-inflammation; nitric oxide; inducible nitric oxide synthase; cyclooxygenase-2

1. Introduction

The genus *Fraxinus* comprises approximately 60 species worldwide. *Fraxinus chinensis* subsp. *rhynchophylla* (Hance) A.E.Murray (Oleaceae) is a deciduous tree that grows widely in Korea and China. The bark of this plant has been used as an antibacterial, analgesic, and anti-inflammatory agent, and is known as the traditional Chinese herbal drug 'Qinpi' in the Chinese Pharmacopoeia. It also has diuretic, anticoagulant, and

CONTACT Ik Soo Lee knifer48@kiom.re.kr; KwangWon Hong hkwon@dongguk.edu
Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2020.1715401.

anti-allergic properties (Kim et al. 1999). Numerous bioactive substances have been isolated from *Fraxinus* species, including coumarins, lignans, flavonoids, secoiridoid glucosides, and simple phenolic compounds, and their pharmacological activities have been verified *in vitro* (Kwon and Kim 1996; Kostova and Iossifova 2007; Si et al. 2008, 2009; Ahn et al. 2013; Hadroug et al. 2019). Further phytochemical studies of *F. chinensis* subsp. *rhynchophylla* bark to search for other anti-inflammatory compounds resulted in the isolation of a new isocoumarin (**1**) and three known compounds (**2**–**4**). Here, we described the isolation and structural elucidation of these compounds and characterized their anti-inflammatory effects by measuring nitric oxide (NO) production and inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) protein expression in lipopolysaccharide (LPS)-induced RAW 264.7 macrophages.

2. Results and discussion

The ethanol extract of *F. chinensis* subsp. *rhynchophylla* bark, which significantly inhibited LPS-induced NO production in RAW 264.7 cells ($IC_{50} = 15.2\,\mu g/mL$), was subjected to a series of chromatographic separation steps guided by NO production inhibitory activity, leading to the isolation of four compounds (**1**–**4**) (Figure 1). Comparing their physicochemical and spectral data with reported data, three known compounds were identified as brevifolin (**2**) (Nawwar et al. 1994), brevifolin carboxylic acid (**3**) (Jiang et al. 2008), and methyl brevifolin carboxylic acid (**4**) (Camacho-Luis et al. 2008).

Compound **1** was obtained as an amorphous yellowish powder, and had a molecular ion peak at *m/z* 629.0774 $[M + Na]^+$ under HRESIMS, corresponding to the molecular formula $C_{26}H_{22}O_{17}$. The IR spectrum of **1** included absorption bands for hydroxy (3254 cm^{-1}), conjugated carbonyl (1665 cm^{-1}), and aromatic (1603 and 1510 cm^{-1}) functional groups. Its UV absorption maxima (225, 278, and 355 nm) further supported the presence of conjugated carbonyl and aromatic ring systems. The ^{1}H-NMR spectrum included characteristic peaks for a galloyl group at δ_H 7.10 (2H, s) (Kaunda and Zhang 2019) and sugar proton signals. In addition, there was an aromatic singlet at δ_H 7.33 and aliphatic ABX-type signals [δ_H 4.58 (1H, dd, $J = 7.5$, 1.8 Hz) and a pair of methylene at δ_H 3.03 (1H, dd, $J = 18.8$, 7.7 Hz) and 2.60 (1H, dd, $J = 18.8$, 1.8 Hz)] attributable to a brevifolin carboxylic acid moiety (Jiang et al. 2008).

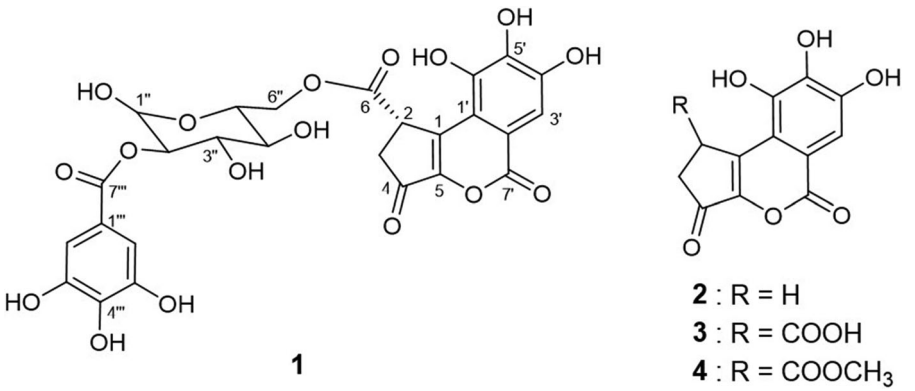


Figure 1. Chemical structures of compounds **1**–**4**.

The ^{13}C-NMR spectrum showed that **1** contained 26 carbons, 13 of which were assigned to galloyl [δ_C 168.6 (C-7‴), 146.9 (C-3‴, 5‴), 140.4 (C-4‴), 121.8 (C-1‴), and 110.9 (C-2‴, 6‴)] and glucosyl [δ_C 91.9 (C-1″), 75.8 (C-2″), 72.8 (C-4″), 72.7 (C-3″), 71.2 (C-5″), and 66.0 (C-6″)] moieties. The remaining 13 carbon signals indicated the presence of an isocoumarin skeleton, consisting of one methylene, one methine, eight quaternary, and three carbonyl carbons. Of these, the two most upfield signals at δ_C 38.9 and 43.0 were assigned to the aliphatic methylenic C-3 and methinic C-2, respectively, whereas the three most downfield signals at δ_C 163.0, 175.0, and 195.9 were attributed to the carbonyl C-7′, C-6, and C-4, respectively. Assignment of the other eight carbons was aided by comparing the chemical shifts with those of the corresponding carbon signals of brevifolin carboxylic acid (**3**). In addition, the *J* value (3.5 Hz) of the anomeric proton at δ_H 5.32 indicated that the glucose moiety was attached in the α-configuration (Roslund et al. 2008). Complete assignment of the chemical shifts of **1** and its substitution pattern was made using various 2 D-NMR techniques, including ^{1}H-^{1}H COSY, HMQC, and HMBC. The HMBC correlations between H-6″ (δ_H 4.35 and 4.50) and C-6 (δ_C 175.0), and between H-2″ (δ_H 4.71) and the carbonyl carbon (δ_C 168.6) of the galloyl group (Figure S1), indicated that the glucose moiety was attached to C-6 of the brevifolin carboxylic acid structure, and the galloyl moiety was linked to C-2″ of glucose. Acid hydrolysis yielded D-glucose as a sugar residue, which was identified by GC analysis of the acid hydrolysate, with gallic acid (**1a**) and brevifolin carboxylic acid (**1b**) as aglycones. The phenylglycine methyl ester (PGME) method (Nagai and Kusumi 1995) was used to determine the absolute configuration of the chiral carboxyl group at C-2 of **1** based on the ^{1}H-NMR data of the (*S*)- and (*R*)-PGME amides of **1b** (Figure S2). A negative $\Delta\delta$ ($\delta_S-\delta_R$) was observed for H-3, and a positive value was observed for H-3′, indicating the *S* configuration of the secondary carboxyl group at C-2 of **1b** (Figure S3). Therefore, the structure of **1** was elucidated as 2-*O*-galloyl 6-*O*-(*S*)-brevifolincarboxyl-α-D-glucopyranoside, and the compound was named fraxicoumarin.

The ability of **1**–**4** to inhibit NO production in LPS-induced RAW 264.7 macrophages was examined. In a toxicity test, these compounds did not affect cell viability at concentrations up to 20 μM (data not shown). Therefore, RAW 264.7 cells were treated with these compounds at concentrations of 1–20 μM. Of the compounds tested, compounds **1** and **3** inhibited LPS-induced NO production in a dose-dependent manner (Figure S4). Treatment with 20 μM **1** and **3** decreased the LPS-induced NO production by about 68% and 61%, respectively, compared with the LPS-treated control, which was comparable to that of L-N^G-monomethyl arginine citrate (L-NMMA; 66% inhibition at 20 μM). Because iNOS and COX-2 are crucial enzymes for NO production (Salvemini et al. 2003), we examined the protein expression of iNOS and COX-2 in LPS-induced RAW 264.7 cells by Western blotting. LPS strongly increased the expression of iNOS and COX-2 protein, and pretreatment with **1** and **3** inhibited the LPS-induced iNOS and COX-2 protein expression in a dose-dependent manner (Figure S4).

This study sought to identify effective anti-inflammatory agents from the bark of *F. chinensis* subsp. *rhynchophylla*, and resulted in the isolation of four isocoumarins (**1**–**4**), including one new compound (**1**). Of these isolates, compounds **1** and **3** dose-dependently inhibited LPS-induced NO production in RAW 264.7 cells. Consistent with

this finding, compounds **1** and **3** also suppressed LPS-induced iNOS and COX-2 protein expression in RAW 264.7 cells. Further studies are needed to understand the specific mechanism(s) involved in the anti-inflammatory effects of these compounds.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured using a JASCO P-2000 digital polarimeter. UV spectra were recorded using a JASCO V-550 UV/VIS spectrometer. IR spectra were recorded on a JASCO 100 IR spectrometer. ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) spectra were obtained using a Bruker DRX-400 spectrometer with tetramethylsilane as an internal standard. 2D-NMR experiments (^{1}H-^{1}H COSY, HMQC and HMBC) were performed on a Bruker Avance 500 NMR spectrometer. HRESIMS was performed using a Shimadzu LCMS-IT-TOF spectrometer. Column chromatography was performed using Diaion HP-20 (Supelco) and YMC-gel ODS-A (12 nm, S-75 μm, YMC). TLC was performed on precoated silica gel 60 F_{254} (0.25 mm; Merck) and RP-18 F_{254s} plates (0.25 mm; Merck). Spots were detected by UV light (254 nm) and spraying of 10% H_2SO_4 followed by heating. Preparative HPLC was performed on a Gilson TRILUTION system with a 321 pump and a UV/Vis 151 detector, and a GX-271 liquid handler (Gilson, Inc.), using a YMC-pack Pro C_{18} (250 × 10 mm, i.d.) column.

3.2. Plant material

Bark of *F. chinensis* subsp. *rhynchophylla* was collected in Gagok-ri, Hwado-eup, Namyangju-si, Gyeonggi-do, Korea, in April 2017, and identified by Prof. Ki Hwan Bae, Chungnam National University, Republic of Korea. A voucher specimen (KIOM-321) has been deposited in the Herbarium of the Korea Institute of Oriental Medicine, Republic of Korea.

3.3. Extraction and isolation

Air-dried *F. chinensis* subsp. *rhynchophylla* stem bark (500 g) was extracted using EtOH (three times, 10 L each time) at room temperature for 7 days, filtered, and concentrated to yield an ethanol extract (48 g). The extract (40 g) was subjected to Diaion HP-20 column chromatography (50 × 10 cm) and eluted with a gradient solvent system consisting of (A) MeOH and (B) water: 100% B (1 L), 80% B (1 L), 60% B (1 L), 40% B (1 L), 20% B (1 L), and 100% A (1 L). The resulting portions from column chromatographic separation were combined into four fractions (A, 5.2 g; B, 4.2 g; C, 3.8 g; D, 6.5 g) based on TLC results. In this bioassay-guided study, the most active column fraction B was chromatographed on a YMC RP-18 column (50 × 6.5 cm) using a MeOH–H_2O gradient solvent system (20:80→100:0) to yield three subfractions (B1–B3). Fraction B1 was further chromatographed on a YMC RP-18 column (50 × 3.5 cm) and eluted with a MeOH–H_2O gradient solvent system (30:70→80:20) to yield compounds **1** (65 mg) and **2** (53 mg). Fraction B2 was purified over a YMC RP-18 column (50 × 2.5 cm) and eluted with a MeOH–H_2O gradient solvent system (40:60→80:20) to

generate compound **3** (38 mg). Chromatography of fraction B3 on a YMC RP-18 column (50 × 2.5 cm) and elution with a MeOH–H_2O gradient solvent system (40:60→90:10) yielded compound **4** (31 mg).

3.3.1. Fraxicoumarin (1)

Amorphous yellowish powder; $[\alpha]_D^{25}$ +4.6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 225 (2.1), 278 (4.0), 355 (1.4) nm; IR (KBr) ν_{max} 3254, 1699, 1665, 1603, 1510, 1447, 1391, 1307, 1199, 1090, 1029 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ_H 7.33 (1H, s, H-3′), 7.10 (2H, s, H-2‴, 6‴), 5.32 (1H, d, *J* = 3.5 Hz, H-1″), 4.71 (1H, dd, *J* = 9.8, 3.5 Hz, H-2″), 4.58 (1H, dd, *J* = 7.5, 1.8 Hz, H-2), 4.50 (1H, dd, *J* = 12.1, 2.1 Hz, H-6″a), 4.35 (1H, dd, *J* = 12.1, 5.6 Hz, H-6″b), 4.08 (1H, m, H-5″), 3.98 (1H, dd, *J* = 9.8, 9.3 Hz, H-3″), 3.35 (1H, dd, *J* = 9.7, 9.3 Hz, H-4″), 3.03 (1H, dd, *J* = 18.8, 7.7 Hz, H-3a), 2.60 (1H, dd, *J* = 18.8, 1.8 Hz, H-3b); ^{13}C NMR (100 MHz, CD_3OD) δ_C 195.9 (C-4), 175.0 (C-6), 168.6 (C-7‴), 163.0 (C-7′), 151.4 (C-4′), 148.1 (C-5), 146.9 (C-3‴, 5‴), 145.3 (C-6′), 141.9 (C-5′), 141.0 (C-1), 140.4 (C-4‴), 121.8 (C-1‴), 116.8 (C-2′), 115.5 (C-1′), 110.9 (C-2‴, 6‴), 109.9 (C-3′), 91.9 (C-1″), 75.8 (C-2″), 72.8 (C-4″), 72.7 (C-3″), 71.2 (C-5″), 66.0 (C-6″), 43.0 (C-2), 38.9 (C-3); HRESIMS *m/z* 629.0774 $[M + Na]^+$ (Calcd for $C_{26}H_{22}O_{17}Na$, 629.0779).

3.4. Acid hydrolysis and identification of sugar

Compound **1** (50 mg) was dissolved in 10% aqueous HCl and refluxed for 3 h. On cooling, the reaction mixture was extracted with EtOAc. After separating the organic layer, the aqueous phase was neutralized with Na_2CO_3 and concentrated. The organic layer was evaporated under reduced pressure, and the residue (30 mg) was purified by preparative RP-HPLC [Gilson TRILUTION system; YMC-pack Pro C_{18} (S-5 μm, 250 × 10 mm) column; ACN/H_2O (20:80); UV detection, 280 nm; flow rate, 4.0 mL/min] to obtain compounds **1a** (8 mg) and **1b** (15 mg). By comparing their spectral data with reported values, **1a** and **1b** were identified as gallic acid and brevifolin carboxylic acid, respectively. A solution of the sugar residue of **1** in 1.5 mL pyridine was added to L-cysteine methyl ester hydrochloride (1.5 mg) and kept at 60 °C for 1 h. Trimethylsilylimidazole (1.5 mL) was added to the mixture and kept at 60 °C for 30 min. The supernatant (4 mL) was then subjected to GC analysis. GC conditions: GC-2010 (Shimadzu) instrument; detector, FID; column, TC-1 capillary column (0.25 mm × 30 m; GL Sciences, Inc.); column temperature, 230 °C; programmed increase, 38 °C/min; carrier gas, N_2 (1 mL/min); injection and detector temperature, 270 °C. The sugar derivative obtained had a retention time of 20.0 min, identical with that of authentic D-glucose.

3.5. Formation of (S)- and (R)-PGME amides

A solution of **1b** (0.03 mmol) and (*S*)-PGME (0.05 mmol) in dry dimethylformamide (1 mL) was treated with benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (0.05 mmol), hydroxybenzotriazole (0.05 mmol), and N-methylmorpholine (0.15 mmol). The reaction mixture was stirred at room temperature for 2 h. Water (1.0 mL) was added to the reaction mixture, which was then extracted with EtOAc. The organic layer was evaporated under reduced pressure, and the residue (3 mg) was

purified by preparative RP-HPLC [Gilson TRILUTION system; YMC-pack Pro C_{18} (S-5 μm, 250 × 10 mm) column; ACN/H_2O (20:80); UV detection, 280 nm; flow rate, 4.0 mL/min] to afford (*S*)-PGME amide **1c** (1.8 mg). Compound **1b** (0.03 mmol) and (*R*)-PGME (0.05 mmol) were treated with the same procedure to afford (*R*)-PGME amide **1d** (1.7 mg).

(*S*)-PGME amide (**1c**): Amorphous yellowish powder; $[\alpha]_D^{25}$ −8.5 (*c* 0.06, MeOH); ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H 7.293 (1H, s, H-3′), 4.367 (1H, dd, *J* = 7.6, 1.8 Hz, H-2), 2.976 (1H, dd, *J* = 18.4, 7.6 Hz, H-3a), 2.491 (1H, dd, *J* = 18.4, 1.8 Hz, H-3b).

(*R*)-PGME amide (**1d**): Amorphous yellowish powder; $[\alpha]_D^{25}$ +7.2 (*c* 0.05, MeOH); ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H 7.287 (1H, s, H-3′), 4.372 (1H, dd, *J* = 7.6, 1.8 Hz, H-2), 3.001 (1H, dd, *J* = 18.4, 7.6 Hz, H-3a), 2.504 (1H, dd, *J* = 18.4, 1.8 Hz, H-3b).

3.6. Cell culture

RAW 264.7 cells were obtained from the American Type Culture Collection (ATCC) and maintained in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (FBS) (Gibco, Thermo Fisher Scientific) and 1% penicillin/streptomycin at 37 °C in a humidified 5% CO_2 incubator.

3.7. Cell proliferation

RAW 264.7 cells were plated in 96-well plates at a density of 1×10^4 cells/well and incubated for 24 h. The medium was replaced with fresh medium containing the indicated concentrations of samples followed by incubation for 24 h. The rate of cell proliferation was measured by MTS assay (Promega) according to the manufacturer's protocol.

3.8. Measurement of NO

RAW 264.7 cells were plated in 12-well plates at a density of 5×10^4 cells/well and incubated for 24 h. The cells were stimulated with 1 μg/mL LPS for 24 h after pretreatment with samples. The supernatants of the cell cultures were harvested and NO production was determined by measuring the amount of nitrite using the Griess reagent system (Promega). Briefly, 100 μL of cell culture medium was mixed with 100 μL of Griess reagent (1% sulfanilamide and 0.1% naphthylenediamide dihydrochloride in 5% H_3PO_4) and incubated at room temperature for 10 min. The absorbance was then measured at 550 nm using a microplate reader (Bio-Rad). Nitrite levels in samples were obtained from a standard $NaNO_2$ curve. L-N^G-monomethyl arginine citrate (Abcam) was used as a positive control.

3.9. Western blot analysis

Cell lysates were prepared using 1 × Laemmli lysis buffer (2.4 M glycerol, 0.14 M Tris-HCl, pH 6.8, 0.21 M sodium dodecyl sulfate, and 0.3 mM bromophenol blue), and boiled for 10 min. The protein concentration was measured using a bicinchoninic acid (BCA) protein assay kit (Pierce Biotechnology). Equal concentrations of protein (20 μg) were separated by 4–20% SDS-PAGE and transferred onto polyvinylidene difluoride

membranes. After blocking, the membranes were incubated with antibodies against β-actin, iNOS, and COX-2 (Cell Signaling Technology). The membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (Cell Signaling Technology) and developed using the enhanced chemiluminescence (ECL) detection system (Amersham Biosciences). Protein activity was determined using an LAS-4000 image analyzer (Fujifilm) and Image Gauge software (Fujifilm).

3.10. Statistical analysis

The results are expressed as means ± standard deviation (SD) from three independent experiments. Statistical significance was assessed using Student's *t*-test with GraphPad 7.0 Prism software (GraphPad).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was supported by a grant (KSN1812080) from the Korea Institute of Oriental Medicine.

References

Ahn JH, Hwang BY, Lee MK. 2013. Simultaneous quantitation of nine constituents of *Fraxinus rhynchophylla* using high performance liquid chromatography-diode array detector. Nat Prod Sci. 19:236–241.

Camacho-Luis A, Gayosso-De-Lucio JA, Torres-Valencia JM, Munoz-Sanchez JL, Alarcon-Hernandez E, Lopez R, Barron BL. 2008. Antioxidant constituents of *Geranium bellum* Rose. J Mex Chem Soc. 52:103–107.

Hadroug A, Belhattab R, Alipieva K, Nedialkov PT. 2019. Isofraxisecoside, a new coumarin-secoiridoid from the stem bark of *Fraxinus xanthoxyloides*. Nat Prod Res. 33(9):1334–1339.

Jiang ZH, Wen XY, Tanaka T, Wu SY, Liu Z, Iwata H, Hirose Y, Wu S, Kouno I. 2008. Cytotoxic hydrolyzable tannins from *Balanophora japonica*. J Nat Prod. 4:719–723.

Kaunda JS, Zhang YJ. 2019. Two new phenolic constituents from the stems of *Euphorbia griffithii*. Nat Prod Bioprospect. 9(6):405–410.

Kim N-Y, Pae H-O, Ko Y-S, Yoo J-C, Choi B-M, Jun C-D, Chung H-T, Inagaki M, Higuchi R, Kim Y-C. 1999. *In vitro* inducible nitric oxide synthesis inhibitory active constituents from *Fraxinus rhynchophylla*. Planta Med. 65(07):656–658.

Kostova I, Iossifova T. 2007. Chemical components of *Fraxinus* species. Fitoterapia. 78(2):85–106.

Kwon YS, Kim CM. 1996. A study on the chemical constituents from leaves of *Fraxinus rhynchophylla*. Kor J Pharmacogn. 27:347–349.

Nagai Y, Kusumi T. 1995. New chiral anisotropic reagents for determining the absolute configuration of carboxylic acids. Tetrahedron Lett. 36(11):1853–1855.

Nawwar MAM, Hussein SAM, Merfort I. 1994. NMR spectral analysis of polyphenols from *Punica granatum*. Phytochemistry. 36(3):793–798.

Roslund MU, Tähtinen P, Niemitz M, Sjöholm R. 2008. Complete assignments of the ^{1}H and ^{13}C chemical shifts and $J_{H,H}$ coupling constants in NMR spectra of D-glucopyranose and all D-glucopyranosyl-D-glucopyranosides. Carbohydr Res. 14:101–112.

Salvemini D, Ischiropoulos H, Cuzzocrea S. 2003. Roles of nitric oxide and superoxide in inflammation. Methods Mol Biol. 225:291–303.

Si CL, Liu Z, Su YF, Kim JK, Bae YS. 2008. Coumarins and secoiridoid glucosides from bark of *Fraxinus rhynchophylla* Hance. Holzforschung. 62(5):553–555.

Si CL, Zhang Y, Zhu ZY, Xu J, Kim JK, Bae YS. 2009. Isolation and structure elucidation of secoiridoid glucosides from *Fraxinus rhynchophylla* leaves. Chem Nat Compd. 45(6): 814–816.