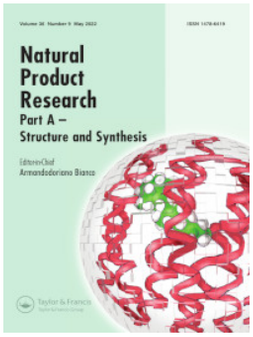


Natural Product Research

Formerly Natural Product Letters

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

Novel phenylpropanoids and isoflavone glycoside are isolated and identified from the carob pods (*Ceratonia siliqua* L.)

Zhi-Tian Peng, Ying-Jie Xia, Takuya Yashiro, Xiong Gao, Tina Ting-Xia Dong, Karl Wah-Keung Tsim & Huai-You Wang

To cite this article: Zhi-Tian Peng, Ying-Jie Xia, Takuya Yashiro, Xiong Gao, Tina Ting-Xia Dong, Karl Wah-Keung Tsim & Huai-You Wang (2022): Novel phenylpropanoids and isoflavone glycoside are isolated and identified from the carob pods (*Ceratonia siliqua* L.), Natural Product Research, DOI: 10.1080/14786419.2022.2076230

To link to this article: https://doi.org/10.1080/14786419.2022.2076230

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2022.2076230

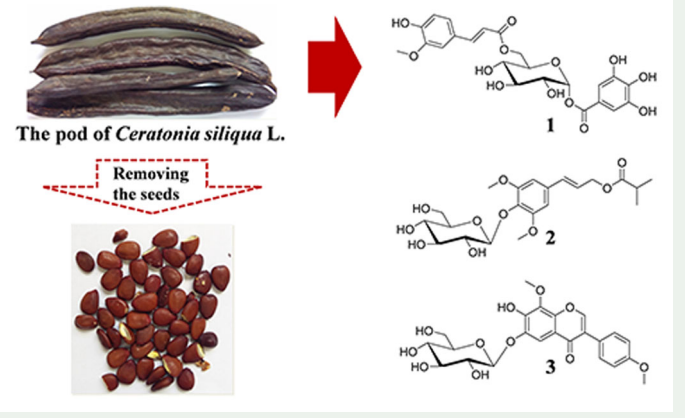
Novel phenylpropanoids and isoflavone glycoside are isolated and identified from the carob pods (*Ceratonia siliqua* L.)

Zhi-Tian Peng[a], Ying-Jie Xia[b], Takuya Yashiro[c], Xiong Gao[b], Tina Ting-Xia Dong[a,b], Karl Wah-Keung Tsim[a,b] and Huai-You Wang[a,d]

[a]Shenzhen Key Laboratory of Edible and Medicinal Bioresources, HKUST Shenzhen Research Institute, Shenzhen, China; [b]Division of Life Science and Center for Chinese Medicine, The Hong Kong University of Science and Technology, Hong Kong, China; [c]Cosmetic Division, Nabocul Cosmetics Company Limited, Tokyo, Japan; [d]Institute of Pharmacy, School of Pharmacy, Henan University, Kaifeng, China

ABSTRACT

Two new phenylpropanoids (**1** and **2**) and one new isoflavone glycoside (**3**), along with nine known compounds (**4** – **12**), were isolated from the pod of *Ceratonia siliqua* L. Their chemical structures were elucidated based on extensive spectroscopic analyses (1 D and 2 D NMR, UV, IR, and HRESIMS) and compared with the literature data. In addition, all isolated compounds were evaluated *in vitro* for inhibitory activity against acetylcholinesterase (AChE). Compounds **4**, **5**, and **12** showed inhibitory activity against acetylcholinesterase (AChE) with IC_{50} values ranging from 15.0 to 50.2 μM.



ARTICLE HISTORY
Received 22 March 2022
Accepted 6 May 2022

KEYWORDS
Ceratonia siliqua L.; phytochemical; phenylpropanoid; isoflavone; acetylcholinesterase inhibition

CONTACT Huai-You Wang wanghy707@163.com

Supplemental data for this article can be accessed online at https://dx.doi.org/10.1080/14786419.2022.2076230.

1. Introduction

The dried bean pod of *Ceratonia siliqua* L., also called carob tree belonging to the genus Leguminosae (Fabaceae) family, is an evergreen tree mainly distributed in Mediterranean region, such as Italy, Spain, Morocco and Portugal (Stavrou et al. 2018). The mature pod of *C. silique* having the length of 10–25 cm consists of about 10% seeds and 90% pulp, and which has been found to contain various constituent, including carbohydrates (40–60%), dietary fibers (27–50%), polyphenols, protein, lipids and minerals, etc. (Rtibi et al. 2017). The categories of polyphenols being identified from *C. silique* are including phenolic acids, flavonoids, tannins, lignans and stilbenes. The pods can be included as animal feed. Due to its high content of sugar and no toxicological sign, the pod of *C. silique* is commonly employed in food industry as locust bean gum, and which is also consumed as candy for children in ancient times or in emergency situations, e.g., war (Owen et al. 2003). The roasted and ground pods are having similarity in flavor to cocoa, and therefore the pod is often being included as a chocolate or cocoa substitute. The production is about 315,000 tons per year in the world.

In addition, the pod of *C. silique* has been employed as folk medicine in the treatment of diarrhoea, heartburn, vomiting, obesity, high cholesterol and pregnancy conditions (Custodio et al. 2011). The pharmacological properties of *C. silique* pod have been proposed in anti-inflammatory, antioxidant, anti-microbial, anti-ulcer, anti-diarrheal and anti-diabetic, anti-depressant, etc (Agrawal et al. 2011; Custódio et al. 2015; Rtibi et al. 2017). Several lines of evidence have shown multiple pharmacological activities of *C. silique* are linking to polyphenols (Owen et al. 2003). In order to discover the biologically active ingredients, the objective of the present study was to investigate the chemical constituents of *C. silique* pod. Two new phenylpropanoids and one new isoflavone glycoside, together with nine known compounds, were identified. Herein, the isolation and structural elucidation of the isolated compounds, as well as their inhibitory activity against acetylcholinesterase (AChE), were described.

2. Results and discussion

From the extract of pod of *C. silique*, twelve compounds were isolated and identified (Figure 1). Compounds **1** – **3** have not been known or identified in *C. siliqua*. The other isolated compounds (**4** – **12**) having known identity were (7*S*,8*R*)-dihydrodehydrodiconiferyl alcohol (**4**) (Park et al. 2011), (+)-syringaresinol (**5**) (Park et al. 2009), (+)-lirioresinol-A (**6**) (Liu et al. 2013), 1-feruloyl-*β*-*D*-glucopyranoside (**7**) (Kim et al. 2011), syringin (**8**) (Kiem et al. 2003), 1′-*O*-coumaroyl-6′-*O*-galloyl-*β*-*D*-glucopyranose (**9**) (Aliotta et al. 1992), trilepisiumic acid (**10**) (Ango et al. 2012), dihydrosyringenin (**11**) (Rustaiyan et al. 1991), pyrocatechol (**12**) (Feng et al. 2011) by comparison of their spectroscopic data with the one reported in literatures.

Compound **1** was obtained as a yellow powder. Its molecular formula was determined to be $C_{23}H_{24}O_{13}$ based on negative-ion HRESIMS (*m/z* 507.1180 [M - H]$^-$), indicating 12 indices of hydrogen deficiency (Supplementary material, Figure S1). The IR spectrum (Supplementary material, Figure S3) showed the presence of hydroxy (3378 cm^{-1}), carbonyl (1700, 1215 cm^{-1}) and aromatic ring (1604, 1516 cm^{-1})

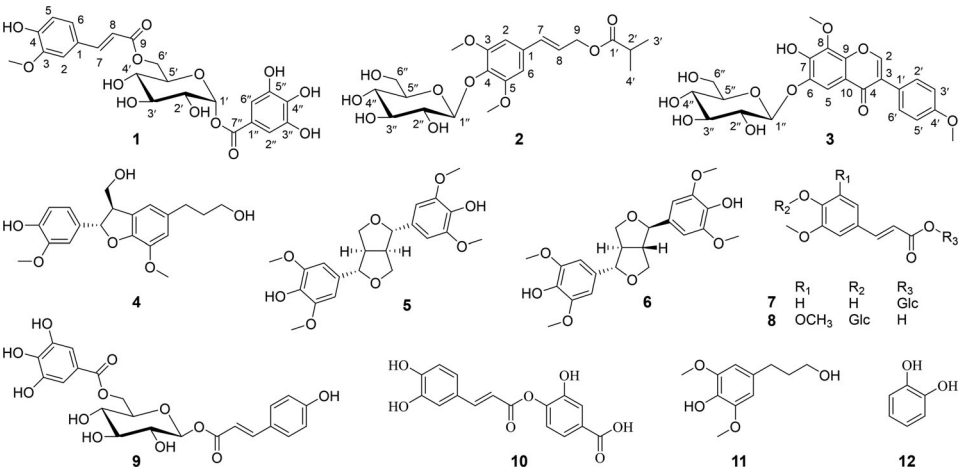


Figure 1. Chemical structure of compounds **1**–**12** from *C. siliqua*.

functionalities. In the ^{1}H NMR spectrum (Supplementary material, Figure S4), two olefinic hydrogens [δ_H 7.62 (1H, d, $J = 15.6$ Hz), 6.39 (1H, d, $J = 15.6$ Hz)], 1,3,4-trisubstituted phenyl group [δ_H 7.18 (1H, s), 7.06 (1H, d, $J = 7.8$ Hz), 6.80 (1H, d, $J = 7.8$ Hz)], 1,3,4,5-tetrasubstituted phenyl group [δ_H 7.14 (2H, s)], one methoxy group [δ_H 3.88 (3H, s)] and one set of glucose residue group [δ_H 5.68 (1H, d, $J = 5.4$ Hz), 4.52 (1H, d, $J = 11.4$ Hz), 4.31 (1H, m), 3.70 (1H, m), 3.51 (2H, m), 3.46 (1H, m)] were observed. The ^{13}C NMR (Supplementary material, Figure S5) spectrum of **1** revealed the occurrence of 23 carbon resonances attributed to two ester carbonyl (δ_C 169.1, 166.9), five oxygenated sp^2 tertiary (δ_C 150.6, 149.3, 146.5 × 2, 140.4), two sp^2 quaternary (δ_C 127.7, 120.6), seven sp^2 methine (δ_C 147.2, 124.2, 116.4, 111.6, 110.6 × 2, 115.2), one methoxy (δ_C 56.4), one set of glucose residue group (δ_C 95.9, 78.0, 76.3, 74.1, 71.3, 64.4) carbons. These NMR spectroscopic data suggested that compound **1** comprised one set of glucose residue group and galloyl group and feruloyl group (Yang et al. 2006).

In the HMBC spectrum (Supplementary material, Figure S7), the correlations from H-2 to C-4/C-6, H-5 to C-1/C-3, H-6 to C-4, H-7 to C-2/C-6/C-9, H-8 to C-1, OCH_3-3 to C-3, along with the NOESY (Supplementary material, Figure S8) correlation of OCH_3-3 with H-2 confirmed the presence of feruloyl group. Subsequently, the galloyl group was verified on the basic of HMBC correlations from H-2″/H-6″ to C-3″/C-4″/C-5″/C-7″ (Supplementary material, Figure S7). Furthermore, the HMBC correlations from H_2-6′ to C-9, H-1′ to C-7″ suggested that feruloyl and galloyl group were connected to the C-6′ and C-1′ of glucose residue, respectively (Supplementary material, Figure S7). The measurement of the optical rotation value of **1** indicated the absolute configuration of glucose was *D*-type. The relative configuration of *D*-glucose was determined as α-type due to the coupling constant of glucose terminal hydrogen δ_H 5.68 (1H, d, $J = 5.4$ Hz, H-1″). Thus, the structure of **1** was established as 1′-*O*-galloyl-6′-*O*-feruloyl-α-*D*-glucopyranose.

Compound **2**, yellow powder, was determined to access to the molecular formula $C_{21}H_{30}O_{10}$, as deduced from positive-ion HRESIMS (*m/z* 465.1983 $[M + Na]^+$), with 7 indices of hydrogen deficiency (Supplementary material, Figure S9). The ^{1}H and ^{13}C NMR data (Supplementary material, Table S2) of **2** were similar to those of syringin

(Kiem et al. 2003) with the exception of an extra isobutyryl group. Analysis of its 1 D and 2 D NMR data indicated that the hydroxy attached to C-9 in syringin was replaced by isobutyryl group in **2**. This deduction was verified by HMBC correlations (Supplementary material, Figure S15) from H_3-3′ to C-1′/C-4′, H-2′ to C-1′/C-4′, H_2-9 to C-1′. The measurement of the optical rotation value of **2** exhibited the presence of *D*-glucose. The relative configuration of *D*-glucose was determined as β-type based on the coupling constant of glucose terminal hydrogen δ_H 4.89 (1H, d, $J = 7.8$ Hz, H-1″). Thus, the structure of **2** was established as 9-isobutyryl syringing.

Compound **3** was obtained as yellow powder, with the molecular formula of $C_{23}H_{24}O_{11}$ as determined on the basis of the positive-ion HRESIMS (*m/z* 499.1266 $[M + Na]^+$) (Supplementary material, Figure S16), demonstrating 12 indices of hydrogen deficiency. The ^{1}H and ^{13}C NMR data (Supplementary material, Table S3) exhibited characteristic resonances of isoflavone skeleton, like 4′,6-dihydroxy-7,8-dimethoxyisoflavone (Wang et al. 2015). The main differences were that a set of glucose residue group was connected to C-6 in **3** instead of hydroxyl, which was supported by the HMBC correlations (Supplementary material, Figure S22) from H-5 to C-4/C-6/C-7/C-8/C-9 and H-1″ to C-6 as well as NOESY correlation (Supplementary material, Figure S23) of H-1″ with H-5. Besides, the *O*-methyl group connected to C-7 and hydroxy attached to C-4′ in 4′,6-dihydroxy-7,8-dimethoxyisoflavone was replaced by hydroxy and *O*-methyl group in **3,** respectively. This deduction was verified by the HMBC correlations from H-5 to C-7 and H-2′/H-6′/OCH_3-4′ to C-4′ (Supplementary material, Figure S22). The measurement of optical rotation value of **3** revealed the presence of *D*-glucose. The relative configuration of *D*-glucose was determined as β-type based on the coupling constant of glucose terminal hydrogen δ_H 5.09 (1H, d, $J = 7.8$ Hz, H-1″). Thus, the structure of **3** was established as 7-hydroxy-8,4′-dimethoxyisoflavone-6-*O*-β- *D*-glucopyranoside.

Compounds **1**–**12** were evaluated *in vitro* for their inhibitory activities against AChE by a modified Ellman's method. BW284C51 (10 μM) was used as a positive control, and AChE inhibition rate was ~61.8%. Compound **5** exhibited robust AChE inhibition having an IC_{50} value at ~15.0 μM, and compounds **4** and **12** showed mild AChE inhibition having IC_{50} values at ~50.2 μM and ~45.7 μM, respectively. The results were shown in Figure S24 in Supplementary material.

3. Experimental

3.1. Plant material

The pod of *C. siliqua* was collected from Republic of Cyprus. The plant material was identified by one of the authors (Dr. Tina Ting-Xia Dong) according to their morphological characteristics. The voucher specimens (CL2018101) were deposited in Shenzhen Key Laboratory of Edible and Medicinal Bioresources, HKUST SRI, China.

3.2. Extraction and isolation

The dried powder pod of *C siliqua* (20.0 kg) after removal of seeds was refluxed with 95% aqueous EtOH (140 L × 2, each for 1.5 h) and 70% aqueous EtOH (140 L, each for

1.5 h), successively. After filtration, the solution was evaporated under reduced pressure to give a residue (12.4 kg) that was then separated by macroporous adsorption resin D101 CC, eluting successively with pure water and 95% aqueous EtOH to obtain the EtOH fraction (462.7 g). The EtOH fraction (460.0 g) was then dissolved in 50% aqueous MeOH and partitioned with petroleum ether and EtOAc. The dried EtOAc extract (135.0 g) was subjected to silica gel CC eluted successively with a gradient of petroleum ether–EtOAc (1:1 to 0:1, v/v) and then CH_2Cl_2–MeOH (7:1 to 1:1, v/v) to yield ten fractions (A–J). Fraction F (18.1 g) was subjected to ODS CC eluted with a gradient of aqueous MeOH from 15% to 100% to afford five sub-fractions (F1–F5). Sub-fraction F4 (4.2 g) was isolated by Sephadex LH-20 column with mobile phase of CH_2Cl_2–MeOH (1:1, v/v), followed by semi-preparative HPLC with mobile phase of isocratic 36% aqueous MeOH to obtain **01** (23.8 mg, t_R 37.14 min). Fraction I (20.0 g) was subjected to ODS CC using a stepwise gradient of aqueous MeOH from 15% to 100% to give five sub-fractions (I1–I5). Sub-fraction I5 (2.7 g) was subjected to Sephadex LH-20 column with mobile phase of CH_2Cl_2–MeOH (1:1, v/v) to give three sub-fractions (I5a–I5c). Compounds **02** (48.3 mg, t_R 27.27 min) and **03** (3.3 mg, t_R 56.42 min) were purified from sub-fractions I5b and I5c by semi-preparative HPLC eluting with isocratic 55% and 49% aqueous MeOH, respectively.

3.2.1. 1′-O-Galloyl-6′-O-feruloyl-α-D-glucopyranose

Yellow powder; UV (MeOH) λ max(logε) 218.0 (4.26), 291.0(4.02), 322.4 (3.95) nm; IR (KBr) ν_{max} 3378, 1700, 1630, 1604, 1516, 1451, 1351, 1215, 1126, 1071, 1032, 766, 580 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table S1; Negative HRESIMS: *m/z* 507.1180 $[M - H]^-$; the original spectra were shown in Figure S1–S8 in Supplementary material.

3.2.2. 9-Isobutyryl syringing

Yellow powder; UV (MeOH) λ max(logε) 211.6 (4.32), 269.2 (3.97) nm; IR (KBr) ν_{max} 3393, 2934, 1731, 1587, 1507, 1465, 1421, 1336, 1243, 1129, 1070, 610 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table S2; Positive HRESIMS: *m/z* 465.1983 $[M + Na]^+$; the original spectra were shown in Figure S9–S15 in Supplementary material.

3.2.3. 7-Hydroxy-8,4′-dimethoxyisoflavone-6-O-β-D-glucopyranoside

Yellow powder; UV (MeOH) λ max(logε) 204.6 (4.07), 264.2 (4.02) nm; IR (KBr) ν_{max} 3422, 1651, 1614, 1588, 1495, 1367, 1280, 1265, 1183, 1056, 1030, 1012, 831 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table S3; Positive HRESIMS: *m/z* 499.1266 $[M + Na]^+$; the original spectra were shown in Figure S16–S23 in Supplementary material.

3.3. AChE inhibition assay

The AChE inhibitory activity of the compounds was determined by the slightly modified Ellman's method (Ellman et al. 1961). In brief, 20 μL of test chemical were added to the reaction mixture with 20 μL of 0.05 U/mL AChE solution (from rat brain lysate) and 20 μL of 0.6 mM 5,50-dithiobis-2-nitrobenzoic acid (DTNB) in 96-well microplates,

pH 7.4. The reaction was initiated by adding 20 μL of 1.2 mM ATCh and then quenched with addition of 20 μL of 4% sodium dodecyl sulfate (SDS) after incubation at 37 °C for 30 min. Absorbance at 405 nm was recorded. BW284C51 was used as positive control. The inhibition rate (%) was calculated as following equation: Inhibition rate (%) = [1 − (OD_S − OD_E)/OD_C] × 100. (The OD_S and OD_C are, respectively, the optical density with and without the test compounds, and OD_E is the optical density without AChE). Inhibition potency of the test compounds was expressed as IC_{50} value, in which all assays were repeated in triplicate.

4. Conclusion

In this study, two new phenylpropanoids (**1** and **2**) and one new isoflavone glycoside (**3**), along with nine known compounds (**4** − **12**), were isolated from the pod of *Ceratonia siliqua* L. Among all these compounds, Compounds **4**, **5**, and **12** showed different levels of inhibitory activity against AChE with IC50 values of ∼50.2, ∼15.0 and ∼45.7 μM, respectively.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by Special project of Foshan University of science and technology in 2019 (FSUST19-SRI10) and Shenzhen Science and Technology Innovation Committee (ZDSYS201707281432317; JCYJ20170413173747440; JCYJ20180306174903174).

ORCID

Huai-You Wang http://orcid.org/0000-0003-1407-9912

References

Agrawal A, Mohan M, Kasture S, Foddis C, Frau MA, Loi MC, Maxia A. 2011. Antidepressant activity of *Ceratonia siliqua* L. fruit extract, a source of polyphenols. Nat Prod Res. 25(4):450–456.

Aliotta G, Molinaro A, Monaco P, Pinto G, Previtera L. 1992. Three biologically active phenylpropanoid glucosides from *Myriophyllum verticillatum*. Phytochemistry. 31(1):109–111.

Ango PY, Kapche DWFG, Kuete V, Ngadjui BT, Bezabih M, Abegaz BM. 2012. Chemical constituents of *Trilepisium madagascariense* (Moraceae) and their antimicrobial activity. Phytochem Lett. 5(3):524–528.

Custodio L, Escapa AL, Fernandes E, Fajardo A, Aligue R, Albericio F, Neng N, Nogueira JMF, Romano A. 2011. Phytochemical profile, antioxidant and cytotoxic activities of the carob tree (*Ceratonia siliqua* L.) germ flour extracts. Plant Foods Hum Nutr. 66(1):78–84.

Custódio L, Patarra J, Alberício F, Neng NR, Nogueira JMF, Romano A. 2015. *In vitro* antioxidant and inhibitory activity of water decoctions of carob tree (*Ceratonia siliqua* L.) on cholinesterases, α-amylase and α-glucosidase. Nat Prod Res. 29(22):2155–2159.

Ellman GL, Courtney KD, Andres V, Feather-Stone RM. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 7:88–95.

Feng WS, Su FY, Zhang XK, Li YJ. 2011. Study on chemical constituents of *Aquilegia yabeana* Kitag. Chin Pharm J. 46(7):496–499.

Kiem PV, Minh CV, Dat NT, Cai XF, Lee JJ, Kim YH. 2003. Two new phenylpropanoid glycosides from the stem bark of *Acanthopanax trifoliatus*. Arch Pharm Res. 26(12):1014–1017.

Kim JS, Kwon YS, Sa YJ, Kim MJ. 2011. Isolation and identification of sea buckthorn (*Hippophae rhamnoides*) phenolics with antioxidant activity and α-glucosidase inhibitory effect. J Agric Food Chem. 59(1):138–144.

Liu Q, Li J, Chai XY, Jiang Y, Tu PF. 2013. Chemical constituents from Qianliang tea. J Chin Pharm Sci. 22(5):427–430.

Owen RW, Haubner R, Hull WE, Erben G, Spiegelhalder B, Bartsch H, Haber B. 2003. Isolation and structure elucidation of the major individual polyphenols in carob fibre. Food Chem Toxicol. 41(12):1727–1738.

Park CH, Kim KH, Lee IK, Lee SY, Choi SU, Lee JH, Lee KR. 2011. Phenolic constituents of *Acorus gramineus*. Arch Pharm Res. 34(8):1289–1296.

Park HB, Lee KH, Kim HK, Lee IK, Noh HJ, Choi SU, Lee KR. 2009. Lignans from the roots of *Berberis amurensis*. Nat Prod Sci. 15(1):17–21.

Rtibi K, Selmi S, Grami D, Amri M, Eto B, El-benna J, Sebai H, Marzouki L. 2017. Chemical constituents and pharmacological actions of carob pods and leaves (*Ceratonia siliqua* L.) on the gastrointestinal tract: a review. Biomed Pharmacother. 93:522–528.

Rustaiyan A, Saberi M, Habibi Z, Jakupovic J. 1991. Melampolides and other constituents from *Furinea leptoloba*. Phytochemistry. 30(6):1929–1932.

Stavrou IJ, Christou A, Kapnissi-Christodoulou CP. 2018. Polyphenols in carobs: a review on their composition, antioxidant capacity and cytotoxic effects, and health impact. Food Chem. 269: 355–374.

Wang Y, Liu CB, Shen QP, Zhang FM, He P, Liu ZH, Zhang HB, Yang XD, Miao MM, Yang GY. 2015. Flavonoids from the leaves of sun cured tobacco and their anti-tobacco mosaic virus activity. Heterocycles. 91(6):1198–1203.

Yang XW, Ma YL, He HP, Wang YH, Di YT, Zhou H, Li L, Hao XJ. 2006. Iridoid constituents of *Tarenna attenuata*. J Nat Prod. 69(6):971–974.