ORIGINAL ARTICLE

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Rec. Nat. Prod. 16:6 (2022) 579-584

records of natural products

A New Alkaloid from *Ormosia hosiei* Hemsl. et Wils

Lijun Zheng [1,#], Linjing Zhang [2,#], Jianyong Huang [1], Xiaoqin Zhang [3], Mingqing Huang [3], Huiyou Xu [2] and Lin Ni [2,*]

[1] *Department of Pharmacy, Fujian Medical University Union Hospital, Fuzhou 350001, People's Republic of China*
[2]*College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou350002, People's Republic of China*
[3]*College of Pharmacy, Fujian Key Laboratory of Chinese Materia Medica, Fujian University of Traditional Chinese Medicine, Fuzhou 350122, People's Republic of China*

(Received Janauary 29, 2022; Revised March 22, 2022; Accepted April 01, 2022)

Abstract: A new alkaloid, hositisine E (**1**) and nine known compounds (**2-10**) were isolated from the seeds of *Ormosia hosiei*Hemsl. et Wils. Compound **1** was identified on the basis of a combination of UV, IR, NMR, CD and HRESIMS data. The structures of the known compounds were determined as isoprunetin (**2**), biochanin A (**3**), ononin (**4**), 4',8-dimethoxy-7-*O*-*β*-*D*-glucopyranosylisoflavone (**5**), sphaerobioside (**6**), ambocin(**7**), rutin (**8**), kaempferol-3-rutinoside (**9**) and narcissin (**10**). In the anti-inflammatory activity assay, compounds **1**-**3**,**7** displayed inhibitory effects against lipopolysaccharide-induced interleukin-6release in RAW264.7 macrophages, exhibiting IC_{50} values of 19.4 – 58.9 μM.

Keywords: *Ormosia hosiei*; cytisine-like alkaloid; hositisine;flavonoid;anti-inflammatory; IL-6.

1. Introduction

The genus *Ormosia* (Leguminosae) has been used for a long time as traditional Chinese medicines and decorations with important medicinal and commercial value [1]. *Ormosiahosiei* Hemsl. et Wils, which is mainly distributed in Fujian, Yunnan, and Sichuan Provinces of People's Republic of China (PRC), has been extensively used in folk medicine to treat rheumatic arthralgia, injuries from falls and irregular menstruations [2]. Previous researches on the *Ormosia*have led to the isolation of alkaloids [3-5],flavonoids [6, 7], lignans [8, 9], triterpenes [5], and volatile oils [10]. And these constituents have been indicated to have anti-inflammatory, anti-tumor, and antifungal effects. In our previous search, some new cytisine-like alkaloids and their biological activities have been investigated from the *Ormosiahosiei* [4, 11]. In our continuing phytochemical study, a new alkaloid (**1**) and nine flavonoids (**2-10**) were isolated (Figure 1) from the seeds of *O.hosiei*, and their anti-inflammatory activities were evaluated.

*Corresponding author: E-mail: nilin_fjau@126.com; Phone: +86-18359772065.
#Lijun Zheng and Linjing Zhang contribute equally to the article.

The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products November-December 2022 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.316.2201.2340
Available online: April 12, 2022

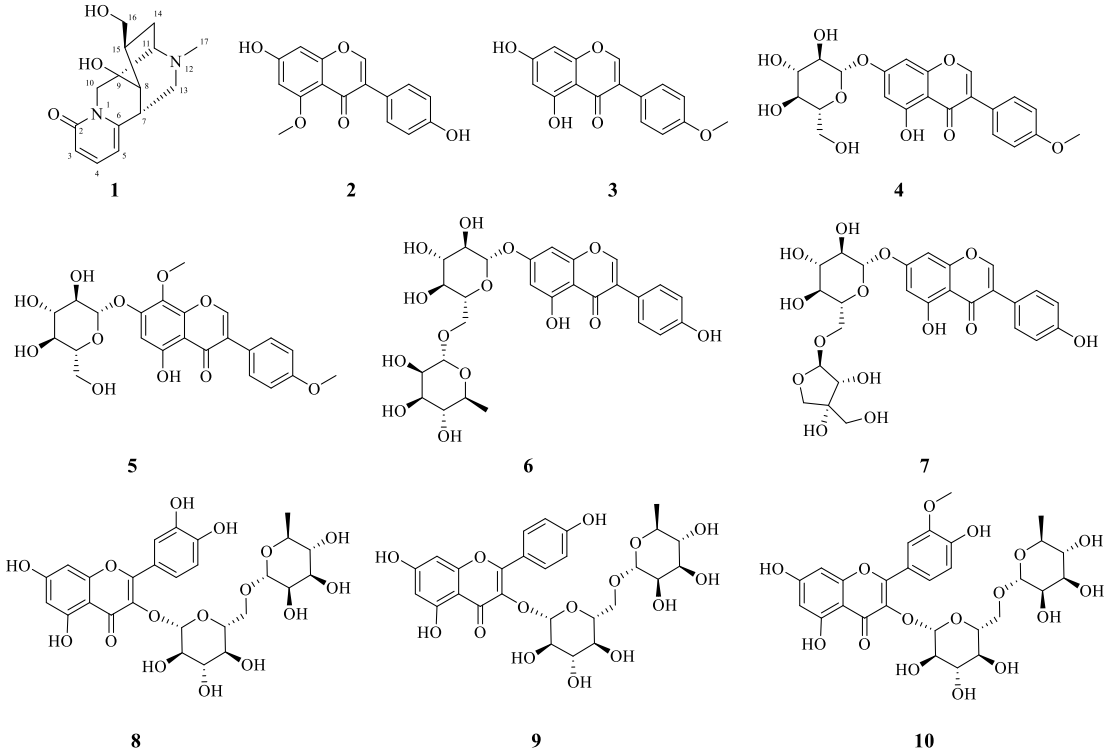

Figure 1 The structures of compounds **1-10**

2. Materials and Methods

2.1. Plant Material

The seeds of the plant *Ormosiahosiei*Hemsl. et Wils were collected from Fuzhou of Fujian province, PRC in Apr. 2017. The voucher specimen (access number: 20170416) was identified by Professor Shuangquan Zou of Fujian Agriculture and Forestry University (FAFU), PRC, and has been storedin College of Plant Protection, FAFU.

2.2. General Experimental Procedures

Chromatography columns were used with polyamide (60-100 mesh, Luqiao Sijia Biochemical Plastic Factory, Taizhou, PRC), PRP-512A resin (Sunflower Technology Development Co., Ltd., Beijing, PRC), Sephadex LH-20 (GE, USA), and silica gel (100-300 mesh, Jiangyou Silica gel Development Co., Ltd., Yantai, PRC). The preparative HPLC was usedby a Shimadzu LC-20AP system with a C_{18}reversed-phase silica gel chromatography column (20 × 250 mm, 5 μm) using an SPD-20A detector. AFourier Transform Infrared Spectrometer worked for IR spectrum (KBr). The 1D and 2D NMR were carried out by a Bruker-Ascend-400MHz instrument. The HR-ESI-MS were measured using a Thermo Scientific LTQ Orbitrap XL.

2.3. Extraction and Isolation

The seeds of *O. hosiei* (2.9 kg) were dried and crushed. The powder of seeds was sequentially extracted with petroleum ether and ethyl acetate at 90 °C for 3 × 2 h. And the extracts were concentrated under reduced pressure to obtain a petroleum ether extract (317.4 g) and an ethyl acetate extract (47.1 g), respectively.The ethyl acetatecrude extract was fractionated by polyamide with EtOH-H_2O (0:1 to 95:5, v/v) to give sixparts (Hd1 – Hd6). Hd1 (13.9 g) was subjected to a PRP-512A column using a EtOH-H_2O (0:1 to 1:0, v/v) gradient to give eight fractions (Hd1.1 – Hd1.8).Hd1.2 (682.4 mg) was subjected to a Sephadex LH-20 column eluted with MeOH and separated using preparative HPLC (CH_3CN-H_2O, 5:95 to 60:40, v/v, 3 mL/min, 300 nm) to obtain four subfractions (Hd1.2.4.1 – Hd1.2.4.4).Hd1.2.4.3 (86.4 mg) was purified by preparative TLC using CH_2Cl_2-EtOH (5:1) mixture to

afford compound **1** (10.3 mg, R_f0.489).Hd3 (812.8 mg) was categorized into four fractions (Hd3.1 – Hd3.4) using a Sephadex LH-20 column with MeOH.Hd3.2 (333.5 mg) was chromatographed by preparative HPLC (CH_3CN-H_2O, 19:81, v/v, 8 mL/min, 254 nm) to generate compound **6** (11.8 mg, t_R 22.412 min),compound **7** (18.1 mg, t_R 23.524 min), compound **8** (10.0 mg, t_R 19.063 min), compound **9** (7.2 mg, t_R 29.706 min) and compound **10** (5.3 mg, t_R 32.153 min).Hd3.3 (164.3 mg) was purified by preparative HPLC (CH_3CN-H_2O, 22:78, v/v, 8 mL/min, 254 nm) toyield compound **4** (21.1 mg, t_R 42.315 min) and compound **5** (11.8 mg, t_R 56.706 min).Hd4 (1.1 g) was purifiedby a Sephadex LH-20 CC with MeOH to obtain compound **2** (26.1 mg).Hd5 (1.3 g) wasseparated and purifiedto obtain compound **3** (49.7 mg) by Sephadex LH-20.

2.4. Spectroscopic Data

Hositisine E (1): white powder, $C_{15}H_{20}N_2O_3$, $[\alpha]_D^{20}$-18.0 (*c* 0.1, MeOH); UV (MeOH)λ_{max}: 230.8, 305.6 nm; IR (KBr)ν_{max}: 3 448, 3 369, 2 923, 1 642 and 1 536 cm^{-1}; CD (MeOH) λ_{max} ($\Delta\varepsilon$): 235.0 (+8.52), 310.0 (-6.37) nm; HRESIMS *m/z* 277.154 1 $[M+H]^+$ (calcd for $C_{15}H_{21}N_2O_3$, 277.154 7); ^{1}H NMR (400 MHz, MeOH-d_4) and ^{13}C NMR (100 MHz, MeOH-d_4): Table 1.

Table 1. The ^{1}H and ^{13}C NMR data of compound **1.**

No.	δ_H (*mult*., *J* in Hz)[a]	δ_C (DEPT)[b]
2	-	165.1(C)
3	6.44(1H, dd, 1.2, 8.8)	116.8(CH)
4	7.48(1H, dd, 6.8, 8.8)	141.7(CH)
5	6.29(1H, dd, 1.2, 6.8)	107.0(CH)
6	-	152.9(C)
7	2.96(1H, overlapped)	44.4(CH)
8	2.12(1H, overlapped)	41.7(CH)
9	-	77.8(C)
10	3.84(1H, d, 15.6); 4.24(1H, d, 15.6)	53.5(CH_2)
11	2.96(1H, overlapped)	73.3(CH)
13	2.44(1H, br d, 11.6); 2.58(1H, dd, 4.4, 11.6)	54.8(CH_2)
14	1.57(1H, dt, 5.6, 14.0); 2.01(1H, dd, 9.2, 14.0)	24.0(CH_2)
15	2.13(1H, overlapped)	45.4(CH)
16	3.69(2H, dd, 4.0, 6.8)	67.2(CH_2)
17	2.15(3H, s)	42.9(CH_3)

[a] In MeOH-d_4 (400 MHz); [b] In MeOH -d_4 (100 MHz).

2.5. Determination of the Production of IL-6

The inhibitory effects on IL-6 of all compounds were evaluated with Lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. Cell cytotoxicity was evaluated by MTT (Sigma, USA) assay. RAW264.7 cells (7×10^5 cells/well) were cultured for 18 h in 96-well plate with Dulbecco's modified Eagle's medium (DMEM) and then the LPS (1 μg/mL) and test materials were added to the cultured cells to stimulate for 24 h. The production of IL-6 was determined by the ELSIA method previously described [12].

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** exhibited a molecular formula as $C_{15}H_{20}N_2O_3$ by HRESIMS at *m/z* 277.154 1 $[M+H]^+$ (calcd for $C_{15}H_{21}N_2O_3$, 277.154 7), which implied an unsaturation degree of 7. The IR spectrum revealed the possible attendance of hydroxyl groups (3 448 and 3 369 cm^{-1})[13] and *α*, *β*-unsaturated lactam (1642 and 1536 cm^{-1}) functionalities [14, 15]. The UV spectrum (230.8, 305.6 nm) showed the typical chromophore of cytisine [16, 17].

The ^{1}H NMR (Figure S1) data of compound **1** displayed a methyl group at δ 2.15 (3H, s) and indicative group signals at δ 7.48 (1H, dd, J = 6.8, 8.8 Hz), 6.44 (1H, dd, J = 1.2, 8.8 Hz), and 6.31 (1H, dd, J = 1.2, 6.8 Hz). The ^{13}C NMR (Figure S2), DEPT (Figure S3) and HSQC (Figure S4) spectroscopic data displayed signals for all 15 carbons, comprising one methyl (δ 42.9), four methylenes (δ 67.2, 54.8, 53.5 and 24.0), seven methines (δ 141.7, 116.8, 107.0, 73.3, 45.4, 44.4 and 41.7) and three quaternary carbons (δ 165.1, 152.9 and 77.8). These NMR data indicated that compound **1** was closely structurally related to hosieine B [3], and the only change is the existof a hydroxyl group at C-9 (δ 77.8) in compound **1**.

The cytisine skeleton was confirmed based on the HMBC correlations (Figure S6) fromH-4 (δ 7.48) to C-2 (δ 165.1) and C-6 (δ 152.9); from H-5 (δ 6.31) to C-3 (δ 116.8), C-6 and C-7 (δ 44.4), from H-10 (δ 4.24 and 3.84) to C-2, C-6, C-8 (δ 41.7), C-9 (δ 77.8) and C-11(δ 73.3); from H-13 (δ 2.44 and 2.58) to C-6; from H-17 (δ 2.15) to C-11 and C-13 (δ 54.8), and the ^{1}H-^{1}H COSY correlations (Figure S5) of H-3 (δ 6.44)/H-4/H-5, H-8 (δ 2.12)/H-7/H-13 and H-11 (δ 2.96)/H-14 (δ 1.07 and 2.57). TheHMBC correlations from H-16 to C-8, C-14 (δ 24.0) and C-15 (δ 45.4)confirmed the C-8 was connected to C-15 and form a bridge ring.Similar to the hosieine B, orientations were assigned by the NOESY correlations (Figure S7) of H-15/H-13 and H-15/H-7.

The absolute configuration of compound **1** was consistent with that of Hosieine B.Due to their similarity (Figure S11), the configurations of **1** wereassigned as 7*R*, 8*R*, 9*R*, 11*R* and 15*R*.

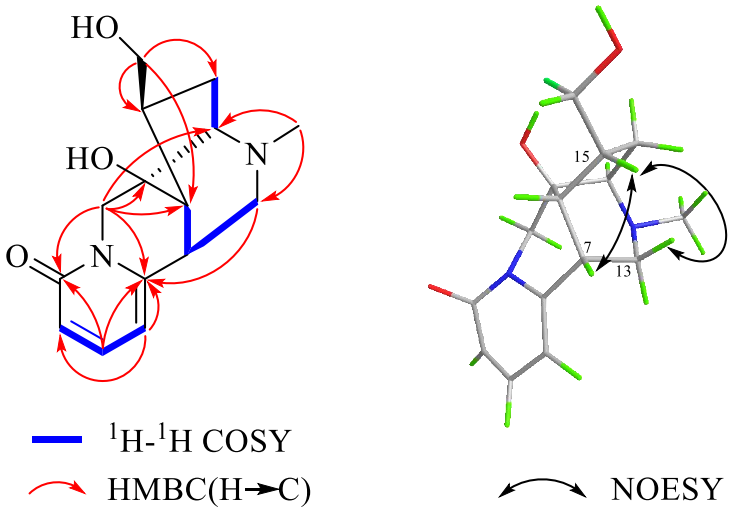

Figure 2 Key HMBC and ^{1}H-^{1}H COSY correlations of compound **1**

The structures of nine isolates (**2**-**10**) were identified as isoprunetin (**2**)[7], biochanin A (**3**)[18], ononin (**4**)[19], 4',8-dimethoxy-7-*O*-β-*D*-glucopyranosylisoflavone(**5**)[20], sphaerobioside (**6**)[21], ambocin(**7**)[22], rutin (**8**)[23], kaempferol-3-rutinoside (**9**)[24] and narcissin (**10**)[23] by detailed analysis of their ^{1}H and ^{13}C NMR spectroscopic data (Table S1 and S2) in comparison with those reported in the references.

3.2. Anti-inflammatory Activity

The anti-inflammatory activities about compounds **1**-**10** were evaluated by measuring their inhibitory effects on IL-6 production on RAW 246.7 cells stimulated by LPS. None of the test materials showed obvious toxicity to RAW 246.7 cells at 0-500 μM. As a result, compounds **1**-**3**, and **7**moderately exhibited inhibitory effect on LPS-induced IL-6 with IC_{50} values of 19.4±1.8, 58.9±5.7, 35.0±3.9 and41.6±3.6 μM, while the IC_{50} of the positive control hydrocortisone was 44.6 ± 5.1 μM. (Table S3).

Acknowledgments

This research is funded by Scientific Research Project of Education Department of Fujian Province (JAT200166), Outstanding-Young Scientific Research Talents Program of Fujian Agriculture and

Forestry University (XJQ202103), and Research Project of National Agricultural Education Steering Committee (2021-NYYB-12).

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

ORCID
Lijun Zheng: 0000-0002-5801-6430
Linjing Zhang: 0000-0001-5996-1816
Jianyong Huang: 0000-0002-0952-7445
Xiaoqin Zhang: 0000-0002-2853-1087
Mingqing Huang: 0000-0001-5752-2932
Huiyou Xu: 0000-0001-8109-8123
Lin Ni: 0000-0001-6118-6724

References

[1] L. J. Zhang, W. J. Zhou, L. Ni, M. Q. Huang, X. Q. Zhang and H. Y. Xu (2021). A review on chemical constituents and pharmacological activities of *Ormosia*, *Chin. Tradit. Herbal. Drugs.* **52**, 4433-4442.
[2] Flora of China Editorial Committee (1994). Flora of China. China Science Press, Beijing.
[3] I. Pouny, M. Batut, L. Vendier, B. David, S. Yi, F. Sautel, P. B. Arimondo and G. Massiot (2014). Cytisine-like alkaloids from *Ormosia hosiei* Hemsl. & E.H. Wilson, *Phytochemistry* **107**, 97-101.
[4] H. Xu, Y. Qiu, J. Chen, Y. Shi, X. Zhang, Q. Song, M. Huang, Z. Wu and L. Ni (2019). Chemical constituents and their activities from the seeds of *Ormosia hosiei*, *Nat. Prod. Commun.* **14**, 1-4.
[5] M. Olivier, J. H. Renault, B. Richard, C. Moretti and M. Zeches-Hanrot (2001). Constituents of the stem bark of *Ormosia wayana*, *Fitoterapia* **72**, 583-584.
[6] L. J. Zhang, Y. X. Quan, L. H. Li, X. Q. Zhang, M. Q. Huang, Y. T. Qiu, L. Ni and H. Y. Xu (2021). Chemical constituents and their anti-inflammatory activity from twigs of *Ormosia hosiei* Hemsl. & E. H. Wilson, *Nat. Prod. Res. Dev.* **33**, 585-591.
[7] Y. T. Qiu, Y. Shi, H. Y. Xu, L. Ni, Q. J. Chen, B. Z. He and H. X. Lu (2018). Flavonoids from the twigs of *Ormosia hosiei* and their anti-fungal activities, *Nat. Prod. Res. Dev.* **30**, 2056-2062.
[8] L. Ni, Y. T. Qiu, Y. Shi, J. X. Chen and H. Y. Xu (2019). Chemical constituents of the roots of *Ormosia hosiei*, *Chem. Nat. Compd.* **55**, 972-974.
[9] S. Feng, J. Hao, Z. Xu, T. Chen and S. X. Qiu (2012). Polyprenylated isoflavanone and isoflavonoids from *Ormosia henryi* and their cytotoxicity and anti-oxidation activity, *Fitoterapia* **83**, 161-165.
[10] D. C. Zhai, J. L. Yao, W. J. Wang, X. Yin, J. Li, X. F. Xi, M. Q. Ren and X. H. Bai (2019).Chemical constituents of the volatile oil from *Ormosia hosiei* leaves and its antioxidant and antimicrobial activity, *Nat. Prod. Res. Dev.* **31**, 814-819.
[11] L. Ni, J. X. Chen, X. Q. Zhang, M. T. Wu, L. J. Zhang, Z. J. Wu, M. Q. Huang and H. Y. Xu (2021). Hositisines A and B, new alkaloids from the stems of *Ormosia hosiei* Hemsl. et Wils, *Nat. Prod. Res.* **35**, 2184-2189.
[12] H. T. Dang, H. J. Lee, E. S. Yoo, P. B. Shinde, Y. M. Lee, J. Hong, D. K. Kim and J. H. Jung (2008). Anti-inflammatory constituents of the red alga *Gracilaria verrucosa* and their synthetic analogues, *J. Nat. Prod.* **71**, 232-240.
[13] X. Wei, Y. Tan, Y. Shi, Y. Yuan and S. Li (2021). A new alkaloid glycoside from the stems of *Zanthoxylum dissitum* Hemsl, *Rec. Nat. Prod.* **15**, 76-81.
[14] Q. M. Pan, Y. H. Li, J. Hua, F. P. Huang, H. S. Wang and D. Liang (2015). Antiviral matrine-type alkaloids from the rhizomes of *Sophora tonkinensis*, *J. Nat. Prod.* **78**, 1683-1688.
[15] H. Zhang, Y.Tan, X.Dong (2021). Two new ecdysteroid glycosides from the rhizomes of *Silene tatarinowii* Regel, *Rec. Nat. Prod.* **15**, 46-52.
[16] J. Zou, L. Zhao, P. Yi, Q. An, L. He, Y. Li, H. Lou, C. Yuan, W. Gu, L. Huang, Z. Hu and X. Hao (2020). Quinolizidine alkaloids with antiviral and insecticidal activities from the seeds of *Sophora tonkinensis* Gagnep, *J. Agric. Food Chem.* **68**, 15015-15026.
[17] B. Danieli, G. Lesma, D. Passarella, A. Sacchetti, A. Silvani and A. Virdis (2004). Total enantioselective synthesis of (-)-cytisine, *Org. Lett.* **6**, 493-496.

[18] I. Polbuppha, V. Suthiphasilp, T. Maneerat, R. Charoensup, T. Limtharakul, S. Cheenpracha, S. G. Pyne and S. Laphookhieo (2021). Macluracochinones A-E, antimicrobial flavonoids from *Maclura cochinchinensis* (Lour.) Corner, *Phytochemistry* **187**, 1-7.

[19] Z. Su, P. Wang, W. Yuan, G. Grant and S. Li (2017). Phenolics from the fruits of *Maclura pomifera*, *Nat. Prod. Commun* .**12**, 1743-1745.

[20] K. Du, M. R. Tian, D. Y.Sheng, M. H. Yuan, Y. Y. Wang, M. Y. Li, Y. L. Zhi, J. L. Ma, G. M. Xue, H. Chen, Y. J. Sun and W. S. Sheng (2021). A new 6a,11a-dehydropterocarpan from rhizomes of *Pueraria lobata*,*Chin. Tradit. Herbal Drugs.* **52**, 4130-4135.

[21] X. Y. Liu, W. Xu, X. W. Yang, P. Zhang, W. Zhao, Y. Gong and N. F. Liu (2020). Isolation and identification of flavonoids from Spatholobi Caulis,*Chin. J. Chin. Mater. Med.* **45**, 1384-1392.

[22] L. Yuan, W. Z. Huang, D. Q. Liang, Y. H. Ma and Z. Z. Du (2015). Study on the flavonoid glycosides from the herb of *Clematis connata* DC, *Chin. Pharm. J.* **50**, 497-501.

[23] Y. B. Cheng, D. M. Zhang and S. S. Yu (2004). Chemical constituents of *Smilax perfoliata*, *Acta Bot. Sin.* **46**, 618-620.

[24] L. C. N. Abraham, T. Masakuni, H. Isao and T. Hajime (2008). Antioxidant flavonoid glycosides from the leaves of *Ficus pumila* L, *Food Chem.* **109**, 415-420.