Chemistry of Natural Compounds, Vol. 47, No. 3, July, 2011 [Russian original No. 3, May–June, 2011]

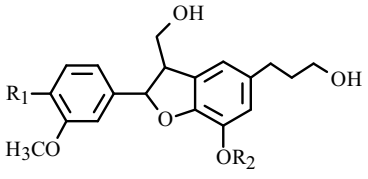
ANTIOXIDANT ACTIVITY OF THE CHEMICAL CONSTITUENTS FROM THE LEAVES OF *Quercus macrocarpa*

Yang-Qing He,[1*] Zhan-Ying Ma,[2] Jian Zhang,[1] Bao-Zhong Du,[1] and Bing-Hua Yao[1*]

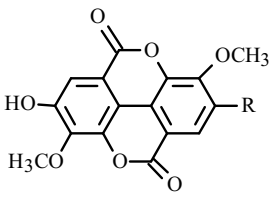
UDC 547.972+547.918

The genus *Quercus* (Fagaceae) comprises about 450 species of evergreen or deciduous shrub or tree, distributed especially in the northern temperate regions in the tropics where they are usually confined to higher altitudes. *Quercus macrocarpa* is one of 110 *Quercus* species native to southwestern China [1]. The extracts of several species of *Quercus* have been found to possess antioxidant, antifungal, antibacterial, and anticancer activities [2–4]. Up to now, no phytochemical investigation has been reported on this plant. In order to find the bioactive secondary metabolites from *Q. macrocarpa*, we investigated its leaves, and here we reported the isolation of 12 known compounds along with their antioxidant activity.

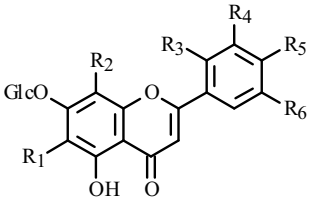
The leaves of *Q. macrocarpa* were collected from Qinling Mountain, Shaanxi Province of China in August of 2007 and identified by Prof. Wang Yifeng the Department of Botany, Northwest Normal University. A Voucher specimen (2007A006) was deposited in the Department of Botany, Northwest Normal University. The air-dried leaves of *Q. macrocarpa* (6.0 kg) were extracted with 90% EtOH three times at room temperature and dried *in vacuo* to give the crude extract. The crude extract (200 g) was sequentially separated on silica gel eluted by step gradients of hexane–ethyl acetate (100:0, 50:50 and 0:100, v/v) and ethyl acetate–methanol mixtures (70:30, 50:50 and 0:100, v/v) to afford six parts (A–F). Fraction B (36 g) was repeatedly subjected to column chromatography on RP-18 silica gel with MeOH–H_2O (2:3, v/v) and Sephadex LH-20 with MeOH to give compounds **1** (9.0 mg), **3** (5.2 mg), **6** (7.1 mg), and **8** (4.6 mg). Fraction C (3.6 g) was chromatographed on silica gel column with petroleum ether–Me_2CO gradient (3:1 to 1:1, v/v) to afford compounds **2** (10.0 mg), **4** (5.0 mg), **5** (6.2 mg), and **7** (8.2 mg). Repeated chromatography of fraction D (2.6 g) on silica gel with petroleum ether–Me_2CO gradient (4:1 to 1:1, v/v) and RP-18 silica gel with MeOH–H_2O gradient (2:3 to 1:1, v/v) afforded compounds **9** (6.0 mg) and **11** (3.5 mg). Fraction E (1.8 g) was submitted to repeated column chromatography over silica gel by eluting with $CHCl_3$–CH_3OH gradient (5:1 to 1:2, v/v) to yield compounds **10** (5 mg) and **12** (3.8 mg).



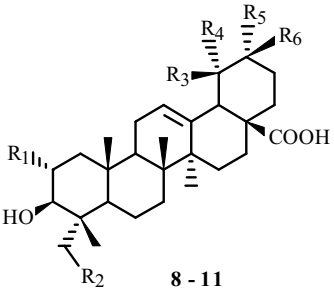
1, 2



3, 4



5, 6



8 - 11

1: R_1 = ORha, R_2 = CH_3; **2:** R = OXyl, R_2 = H; **3:** R = OXyl; **4:** R = ORha; **5:** R_1 = R_2 = R_3 = R_6 = H, R_4 = R_5 = OH
6: R_1 = R_4 = R_5 = R_6 = H, R_2 = OCH_3, R_3 = OH; **7:** R_2 = R_3 = H, R_1 = R_4 = R_5 = R_6 = OH
8: R_1 = R_4 = OH, R_2 = R_3 = H, R_5 = R_6 = CH_3; **9:** R_1 = R_2 = R_4 = OH, R_3 = H, R_5 = R_6 = CH_3
10: R_1 = R_2 = R_3 = R_4 = H, R_5 = R_6 = CH_3; **11:** R_1 = R_2 = R_4 = R_6 = H, R_5 = R_3 = CH_3

1) Department of Applied Chemistry, Xi′an University of Technology, Xi′an 710054, P. R. China, fax: +86 29 82066361, e-mail: hyq7978863@163.com; bhyao@xaut.edu.en; 2) Department of Chemistry, Xianyang Normal University, Xianyang 712000, P. R. China. Published in Khimiya Prirodnykh Soedinenii, No. 3, pp. 420–421, May–June, 2011. Original article submitted February 4, 2010.

TABLE 1. Scavenging Activity of Isolated Compounds and Ascorbic Acid against DPPH Radical

Compound	SC_{50}, μM	Compound	SC_{50}, μM
1	32.2 ± 0.8	**7**	9.2 ± 0.3
2	30.6 ± 0.6	**8**	2.0 ± 0.6
3	67.8 ± 0.5	**9**	25.6 ± 0.2
4	76.6 ± 0.7	**10**	7.5 ± 0.7
5	10.2 ± 0.2	**11**	9.6 ± 0.6
6	13.2 ± 0.9	Ascorbic acid	41.6 ± 0.5

The values are the average of three determinations (± S.D.).

The compounds were identified as icariside E4 (**1**) [5], junipercomnoside A (**2**) [5], 3,3′-di-*O*-methylellagic acid-4′-*O*-*β*-D-xylopyranoside (**3**) [6], 3,3′-di-*O*-methylellagic acid-4′-*O*-*α*-L-rhamnopyranoside (**4**) [7], luteolin-7-*O*-*β*-D-glucoside (**5**) [8], 5,7,2′-trihydroxy-8-methoxyflavone-7-*O*-*β*-D-glucopyranoside (**6**) [9], 5,6,7,3′,4′,5′-hexahydroxyflavone-7-*O*-*β*-D-glucopyranoside (**7**) [10], arjunic acid (**8**) [11], arjungenin (**9**) [11], oleanolic acid (**10**) [12], ursolic acid (**11**) [13], and *β*-sitosterol (**12**) [14] by comparison of their NMR and MS spectroscopic data with those reported in the literature. All these compounds were obtained from this plant for the first time.

For the evaluation of the antioxidant capacity of compounds **1**–**11**, a DPPH assay was performed as described previously (ascorbic acid used as positive control) [14]. The data concerning the free radical scavenging activity of tested samples, expressed as SC_{50} (the concentration of sample required to scavenge 50% of DPPH radicals), are reported in Table 1. The highest values were obtained for compounds **5**–**7**, **8**, **10**, and **11**. Flavonoids **5**–**7** displayed stronger DDPH radical scavenging activity than ascorbic acid in the order **7** > **5** > **6**. This result is in accord with the finding that the more B-ring hydroxyl groups attached to the molecule, the stronger the antioxidative activity [14]. Lower activity was found for compounds **1**, **2**, and **9**, whereas compounds **3** and **4** turned out to be inactive.

ACKNOWLEDGMENT

This work was supported by the Scientific Research Program Funded by Shaanxi Provincial Education Department (2010JK749).

REFERENCES

1. Institute of Botany, Chinese Academy of Sciences & Institute of Materia Medica, *Flora Reipublicae Popularis Sinicae*. Science Press, Beijing, Vol. **22**, 1979, p. 213.
2. A. L. Adonizio, K. Downum, B. C. Bennett, and K. Matllee, *J. Ethnopharmacol.*, **105**, 427 (2006).
3. D. Walters, B. Meurer-Grimes, and I. Rovira, *FEMS Microbiol Lett.*, **201**, 255 (2001).
4. H. M. Ge, Y. Shen, C. H. Zhu, S. H. Tan, H. Ding, Y. C. Song, and R. X. Tan, *Phytochemistry*, **69**, 571 (2008).
5. T. Nakanishi, N. Iida, Y. Inatomi, H. Murata, A. Inada, J. Murata, F. A. Lang, M. Iinuma, and T. Tanaka, *Phytochemistry*, **65**, 207 (2004).
6. X. H. Shi, X. L. Du, and L. Y. Kong, *China J. Chin. Mater. Med.*, **31**, 1503 (2006).
7. S. H. Wu, Y. M. Shen, Y. W. Chen, Z. Y. Li, L. T. Yang, and S. L. Li, *Chem. Nat. Comp.*, **45**, 536 (2009).
8. Y. Xu and J. Y. Liang, *J. China Pharm. Univ.*, **36**, 411 (2005).
9. Y. Miyaichi, E. Hanamitsu, H. Kiu, and T. Tomimori, *Chem. Pharm. Bull.*, **54**, 435 (2006).
10. Y. H. Meng, A. J. Krzysiak, M. J. Durako, J. I. Kunzelman, and J. L. Wright, *Phytochemistry*, **69**, 2603 (2008).
11. Y. P. Shan and L. Y. Kong, *Chin. J. Nat. Med.*, **4**, 271 (2006).
12. K. Kamiya, K. Yoshioka, Y. Saiki, A. Ikuta, and T. Satake, *Phytochemistry*, **44**, 141 (1997).
13. L. J. Tian and F. E. Wu, *Nat. Prod. Res. Dev.*, **15**, 502 (2003).
14. Q. Liu, Y. J. Zhang, C. R. Yang, and M. Xu, *J. Agric. Food Chem.*, **57**, 586 (2009).