**Suppl. 2.** **Original spectra of HPLC-MS spectra**

**Antioxidation and Cytoprotection of Acteoside and its Derivatives: Comparison and Mechanistic Chemistry**

Xican Li 1,2, \*,†, Yulu Xie 1,2,†, Ke Li 3,4, Aizhi Wu 1,2, \*, Hong Xie 1,2, Qian Guo 1,5, Penghui Xue 1, Yerkingul Maleshibek 1, Wei Zhao 6, Jiasong Guo 7, and Dongfeng Chen 3,4

1 School of Chinese Herbal Medicine; Guangzhou University of Chinese Medicine, Guangzhou 510006, China. E-mails: xieyulu1900@163.com (Y.X.); xiehongxh1@163.com (H.X.); 15622178307@163.com (Q.G.); 15228738137@163.com (P.X.); pandiphd@163.com (Y.M.);

2 Innovative Research & Development Laboratory of TCM; Guangzhou University of Chinese Medicine, Guangzhou 510006, China.

3 School of Basic Medical Science, Guangzhou University of Chinese Medicine, Guangzhou, China, 510006; E-mails: [ys1090992678@163.com](mailto:ys1090992678@163.com) (K.L.)

4 The Research Center of Basic Integrative Medicine, Guangzhou University of Chinese Medicine, Guangzhou, China, 510006. E-mail: chen888@gzucm.edu.cn (D.C.)

5 School of Basic Medical Science; Guangdong Pharmaceutical University, Guangzhou, China, 510007.

6 ZhongshanSchool of Medicine; Sun Yat-sen University, No.74 Zhongshan Road. 2, Guangzhou, 510080, China.

7 Department of Histology and Embryology, Southern Medical University, Guangzhou, 510515, China.

\*Correspondence author:

E-mail：[lixican@126.com](mailto:lixican@126.com) [wuaizhi@gzucm.edu.cn](mailto:wuaizhi@gzucm.edu.cn)

Homepage:[www.researchgate.net/profile/Xican\_Li](http://www.researchgate.net/profile/Xican_Li)

Note:

The supplemental materials are involved in four compounds, i.e. acteoside, forsythoside B, poliumoside, and caffeic acid. These materials tried to demonstrate that, each of acteoside, forsythoside B, and poliumoside have no RAF reaction with DPPH•, and no dimerization reaction; However, caffeic acid happen a dimerization reaction.

The determining conditions are detailed in Section 4.5 of main text.



Fig. S2.1 [Total ion chromatogram](http://www.baidu.com/link?url=CF2kwqGsxR0oJevz0eMOkaKlSp5m3CP5azTuAPtPYQywgNd79FAccvr91aMdqMonPznhus0HQnaImc9imgKjcZGfDHgKz2a0BLH0fQFGHU8hiKdjXP7HKk5Oo13Brtbt) of **acteoside**



Fig. S2.2 [Total ion chromatogram](http://www.baidu.com/link?url=CF2kwqGsxR0oJevz0eMOkaKlSp5m3CP5azTuAPtPYQywgNd79FAccvr91aMdqMonPznhus0HQnaImc9imgKjcZGfDHgKz2a0BLH0fQFGHU8hiKdjXP7HKk5Oo13Brtbt) of DPPH•



Fig. S2.3 [Ion chromatogram of](http://www.baidu.com/link?url=CF2kwqGsxR0oJevz0eMOkaKlSp5m3CP5azTuAPtPYQywgNd79FAccvr91aMdqMonPznhus0HQnaImc9imgKjcZGfDHgKz2a0BLH0fQFGHU8hiKdjXP7HKk5Oo13Brtbt)  reaction product of **acteoside** with DPPH• extracted by C47H46N5O21

The reaction was conducted by mixing acteoside and DPPH• (1:2, molar ratio) . The reaction mixture was incubated for 24h. The product mixture was analyzed using UPLC−ESI−Q−TOF−MS/MS technology. For the convenience of determination, the corresponding chemical formula of acteoside-DPPH• adduct was extracted by the software. The corresponding chemical formula was C47H46N5O21 (acteoside is C29H36O15; DPPH• is C18H12N5O6. Thus, [covalent](http://www.baidu.com/link?url=_NXpqH1Jy8-wYHBhYKz4OjCAE3JoN555TWPHLpcBZLLZRIaurfOQAmGBjgiSpGmKGQT_S1R2aAwmy80CmAIxxeLdfEfJ5extKEgaPUc1uAm) adduct should be C47H46N5O21). However, the spectra give no corresponding peak (The intensity in [Y-axis](http://www.baidu.com/link?url=Z6HvnDoXbWTkMqe9qMuC1GgntWV4Hi8GWrpEWlWrktwYOlPO_3DubKxLBZb7zWwWqIqkHfSl0rRVa4LyjMMtjvX_Qg7AKe1m3InrGmMl2Au) is negligible)



Fig. S2.4 [Ion chromatogram of](http://www.baidu.com/link?url=CF2kwqGsxR0oJevz0eMOkaKlSp5m3CP5azTuAPtPYQywgNd79FAccvr91aMdqMonPznhus0HQnaImc9imgKjcZGfDHgKz2a0BLH0fQFGHU8hiKdjXP7HKk5Oo13Brtbt)  reaction product of **acteoside** dimer extracted by C58H70O30

The reaction and determination conditions were described in the footnote of Fig. S2.3. For the convenience of determination, the corresponding chemical formula of acteoside-acteoside dimer was extracted by the software. The corresponding chemical formula was C58H70O30 (acteoside is C29H36O15. Thus, dimeric acteoside [covalent](http://www.baidu.com/link?url=_NXpqH1Jy8-wYHBhYKz4OjCAE3JoN555TWPHLpcBZLLZRIaurfOQAmGBjgiSpGmKGQT_S1R2aAwmy80CmAIxxeLdfEfJ5extKEgaPUc1uAm) adduct should be C58H70O30). However, the spectra give no corresponding peak. (The intensity in [Y-axis](http://www.baidu.com/link?url=Z6HvnDoXbWTkMqe9qMuC1GgntWV4Hi8GWrpEWlWrktwYOlPO_3DubKxLBZb7zWwWqIqkHfSl0rRVa4LyjMMtjvX_Qg7AKe1m3InrGmMl2Au) is negligible)



Fig. S2.5 [Total ion chromatogram](http://www.baidu.com/link?url=CF2kwqGsxR0oJevz0eMOkaKlSp5m3CP5azTuAPtPYQywgNd79FAccvr91aMdqMonPznhus0HQnaImc9imgKjcZGfDHgKz2a0BLH0fQFGHU8hiKdjXP7HKk5Oo13Brtbt) of **forsythoside B**



Fig. S2.6 [Total ion chromatogram](http://www.baidu.com/link?url=CF2kwqGsxR0oJevz0eMOkaKlSp5m3CP5azTuAPtPYQywgNd79FAccvr91aMdqMonPznhus0HQnaImc9imgKjcZGfDHgKz2a0BLH0fQFGHU8hiKdjXP7HKk5Oo13Brtbt) of DPPH•



Fig. S2.7 [Ion chromatogram of](http://www.baidu.com/link?url=CF2kwqGsxR0oJevz0eMOkaKlSp5m3CP5azTuAPtPYQywgNd79FAccvr91aMdqMonPznhus0HQnaImc9imgKjcZGfDHgKz2a0BLH0fQFGHU8hiKdjXP7HKk5Oo13Brtbt)  reaction product of **forsythoside B** with DPPH• extracted by C52H54N5O25. The reaction was conducted by mixing acteoside and DPPH• (1:2, molar ratio) . The reaction mixture was incubated for 24h. The product mixture was analyzed using UPLC−ESI−Q−TOF−MS/MS technology. For the convenience of determination, the corresponding chemical formula of forsythoside B-DPPH• adduct was extracted by the software. The corresponding chemical formula was C52H54N5O25 (forsythoside B is C34H44O19; DPPH• is C18H12N5O6. Thus, [covalent](http://www.baidu.com/link?url=_NXpqH1Jy8-wYHBhYKz4OjCAE3JoN555TWPHLpcBZLLZRIaurfOQAmGBjgiSpGmKGQT_S1R2aAwmy80CmAIxxeLdfEfJ5extKEgaPUc1uAm) adduct should be C52H54N5O25). However, the spectra give no corresponding peak. (The intensity in [Y-axis](http://www.baidu.com/link?url=Z6HvnDoXbWTkMqe9qMuC1GgntWV4Hi8GWrpEWlWrktwYOlPO_3DubKxLBZb7zWwWqIqkHfSl0rRVa4LyjMMtjvX_Qg7AKe1m3InrGmMl2Au) is negligible)



Fig. S2.8 [Ion chromatogram of](http://www.baidu.com/link?url=CF2kwqGsxR0oJevz0eMOkaKlSp5m3CP5azTuAPtPYQywgNd79FAccvr91aMdqMonPznhus0HQnaImc9imgKjcZGfDHgKz2a0BLH0fQFGHU8hiKdjXP7HKk5Oo13Brtbt)  reaction product of **forsythoside B** dimerextracted by C68H86O38.

The reaction and determination conditions were described in the footnote of Fig. S2.7. However, the chemical formula C68H86O38 was extracted for detection of forsythoside B-forsythoside B dimer (forsythoside B is C34H44O19. Thus, the dimer should be C68H86O38). However, the spectra give no corresponding peak. (The intensity in [Y-axis](http://www.baidu.com/link?url=Z6HvnDoXbWTkMqe9qMuC1GgntWV4Hi8GWrpEWlWrktwYOlPO_3DubKxLBZb7zWwWqIqkHfSl0rRVa4LyjMMtjvX_Qg7AKe1m3InrGmMl2Au) is negligible)



Fig. S2.9 [Total ion chromatogram](http://www.baidu.com/link?url=CF2kwqGsxR0oJevz0eMOkaKlSp5m3CP5azTuAPtPYQywgNd79FAccvr91aMdqMonPznhus0HQnaImc9imgKjcZGfDHgKz2a0BLH0fQFGHU8hiKdjXP7HKk5Oo13Brtbt) of **poliumoside**



Fig. S2.10 Total ion chromatogram of **DPPH•**



Fig. S2.11 [Ion chromatogram of](http://www.baidu.com/link?url=CF2kwqGsxR0oJevz0eMOkaKlSp5m3CP5azTuAPtPYQywgNd79FAccvr91aMdqMonPznhus0HQnaImc9imgKjcZGfDHgKz2a0BLH0fQFGHU8hiKdjXP7HKk5Oo13Brtbt)  reaction product of **poliumoside** with DPPH• extracted by C52H54N5O25. The reaction was conducted by mixing poliumoside and DPPH• (1:2, molar ratio) . The reaction mixture was incubated for 24h. The product mixture was analyzed using UPLC−ESI−Q−TOF−MS/MS technology. For the convenience of determination, the corresponding chemical formula of poliumoside-DPPH• adduct was extracted by the software. The corresponding chemical formula was C53H56N5O25 (poliumoside is C35H46O19; DPPH• is C18H12N5O6. Thus, [covalent](http://www.baidu.com/link?url=_NXpqH1Jy8-wYHBhYKz4OjCAE3JoN555TWPHLpcBZLLZRIaurfOQAmGBjgiSpGmKGQT_S1R2aAwmy80CmAIxxeLdfEfJ5extKEgaPUc1uAm) adduct should be C53H56N5O25). However, the spectra give no corresponding peak. (The intensity in [Y-axis](http://www.baidu.com/link?url=Z6HvnDoXbWTkMqe9qMuC1GgntWV4Hi8GWrpEWlWrktwYOlPO_3DubKxLBZb7zWwWqIqkHfSl0rRVa4LyjMMtjvX_Qg7AKe1m3InrGmMl2Au) is negligible)



Fig. S2.12 [Ion chromatogram of](http://www.baidu.com/link?url=CF2kwqGsxR0oJevz0eMOkaKlSp5m3CP5azTuAPtPYQywgNd79FAccvr91aMdqMonPznhus0HQnaImc9imgKjcZGfDHgKz2a0BLH0fQFGHU8hiKdjXP7HKk5Oo13Brtbt)  reaction product of **poliumoside** dimerextracted by C70H90O38.

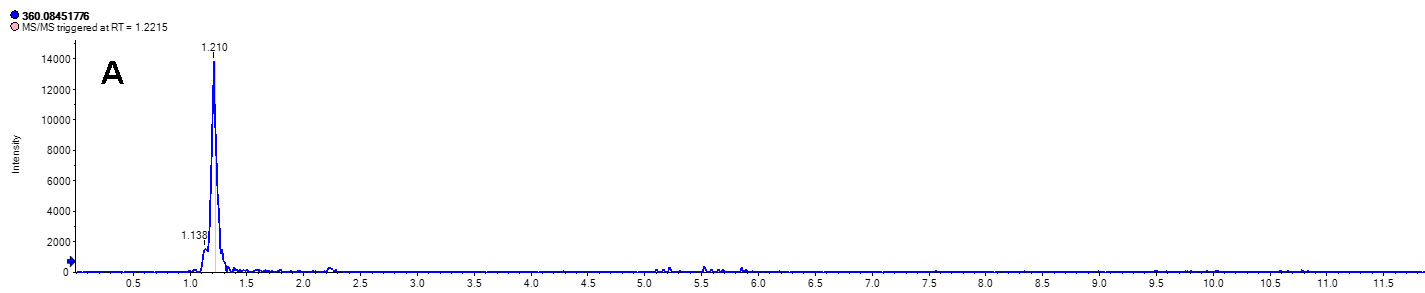
The reaction and determination conditions were described in the footnote of Fig. S2.1. However, the chemical formula C70H90O38 was extracted for detection of poliumoside-poliumoside dimer (poliumoside is C35H46O19. Thus, the dimer should be C70H90O38). However, the spectra give no corresponding peak. (The intensity in [Y-axis](http://www.baidu.com/link?url=Z6HvnDoXbWTkMqe9qMuC1GgntWV4Hi8GWrpEWlWrktwYOlPO_3DubKxLBZb7zWwWqIqkHfSl0rRVa4LyjMMtjvX_Qg7AKe1m3InrGmMl2Au) is negligible)



Fig. S2.13 [Total ion chromatogram](http://www.baidu.com/link?url=CF2kwqGsxR0oJevz0eMOkaKlSp5m3CP5azTuAPtPYQywgNd79FAccvr91aMdqMonPznhus0HQnaImc9imgKjcZGfDHgKz2a0BLH0fQFGHU8hiKdjXP7HKk5Oo13Brtbt) of **caffeic acid**



Fig. S2.14 Total ion chromatogram of **DPPH•**



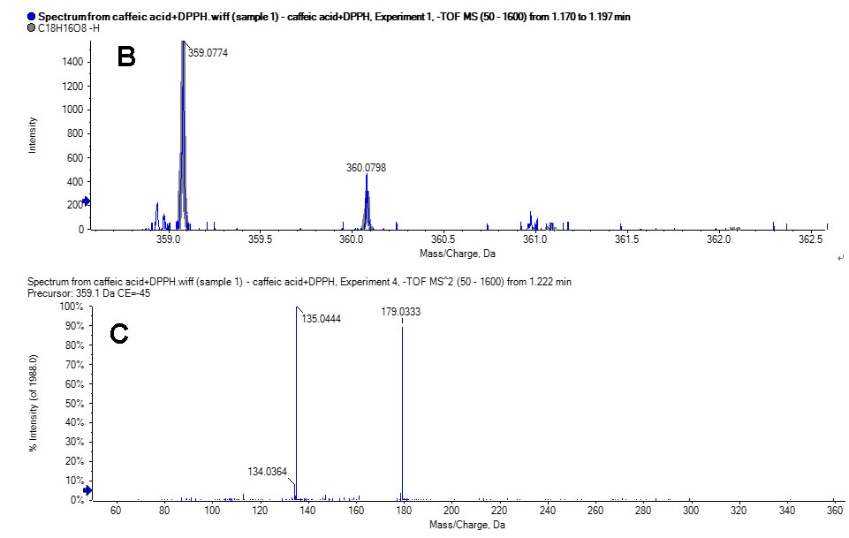


Fig. S2.15 Ion chromatogram of reaction product of **caffeic acid** dimerextracted by C18H14O8. The reaction was conducted by mixing caffeic acid and DPPH• (1:2, molar ratio) . The reaction mixture was incubated for 24h. The product mixture was analyzed using UPLC−ESI−Q−TOF−MS/MS technology. For the convenience of determination, the corresponding chemical formula of caffeic acid-caffeic acid dimer was extracted by the software. The corresponding chemical formula was C18H14O8 (caffeic acid is C9H8O4. Thus, the dimer should be C18H14O8).

As seen in the Fig. S2.15A, the spectra give a evident corresponding peak at 1.210 min.

The peak at 1.210 min yielded a molecular ion peak *m/z* 359-360 (Fig. S2.15B)

The molecular ion peak in Fig. S2.15B was further broken to give rise to secondary MS spectra *m/z* 179, 135 in Fig. S2.15C. In terms of literatures (Brand-Williams, W., M. E. Cuvelier, C. Berset, Use of a Free Radical Method to Evaluate Antioxidant Activity. Lebensm.-Wiss. u.-Technol, 1995. 28: p. 25-30; Foti, M.C., Antioxidant properties of phenols. J Pharm Pharmacol, 2007. 59(12): p. 1673-85), the pathway and MS spectra elucidation of one of RAF product can be described as the following:



**In a word, Fig. S2.15A, B, and C display that, caffeic acid happened dimerization reaction when mixed with DPPH•.**