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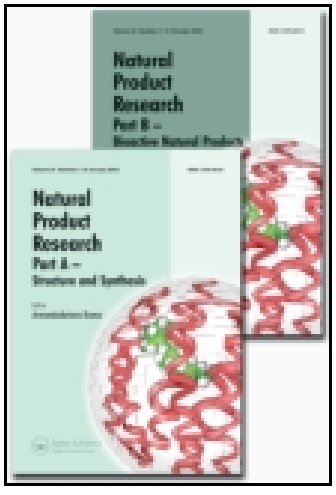

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Antioxidants from Gerbera piloselloides: an ethnomedicinal plant from southwestern China

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SHORT COMMUNICATION

Antioxidants from *Gerbera piloselloides*: an ethnomedicinal plant from southwestern China

Jianjun Wang[a], Vanya Petrova[b], Shi-Biao Wu[b], Mingjing Zhu[a], Edward J. Kennelly[ab] and Chunlin Long[ac]*

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Gerbera piloselloides is a very important ethnobotanical and ethnomedicinal plant used by indigenous peoples in southwestern China. Ten compounds were obtained using activity-guided isolation, including a parasorbosid derivative, two caffeic acid derivatives, two coumarins and five flavonoids, and identified from the whole plant of *G. piloselloides*. This is the first report of compound **5** as natural product. Six compounds were reported in the *Gerbera* genus for the first time. The antioxidant activity of all the compounds was evaluated by using ABTS assay, and the chemotaxonomic implication of this study was also discussed.

Keywords: *Gerbera piloselloides*; antioxidant; ABTS; TPC; chemotaxonomy

1. Introduction

Gerbera piloselloides (L.) Cass. (Asteraceae) is a perennial herb distributed in open areas and forest margins. The genus *Gerbera* includes about 70 species distributed from Africa to eastern Asia with 10 species found in China, mainly in its southwestern region (Tourjee et al. 1994). Traditionally, in China, people used *G. piloselloides* for clearing heat and detoxifying (Wu & Peng 2002). The Zhuang people of Jingxi County, southwest China, liked to use it as antitussive in the form of an herbal tea with honey, and spice in preparing meat and wine owing to its good smell. Although this plant has an important usage in traditional medicine, its taxonomy remains disputed and inconsistent.

Previous studies on *G. piloselloides* reported the presence of coumarins, flavonoids, phenols, polysaccharides, succinic acid, isoborneol and β-sitosterol, and also revealed that its compounds and extracts possessed antitussive activity (Halim et al. 1980; Gu et al. 1989; Xiao & Ding 2002; Xiao et al. 2003; She et al. 2004). Interestingly, this study partially proved the traditional knowledge of the Zhuang people in using this plant as effective antitussive, and also supported further foundation for the taxonomy of two sister genera, *Gerbera* and *Piloselloides*.

2. Results and discussion

2.1. *Phytochemistry and antioxidant activity*

Using activity-guided method, 10 compounds (Figure 1), caffeic acid (**1**), 3,4-dicaffeoylquinic acid (**2**), marmesinin (**3**) (Basnet et al. 1996), 7,8-dihydroxycoumarin (**4**) (Chawla et al. 1980),

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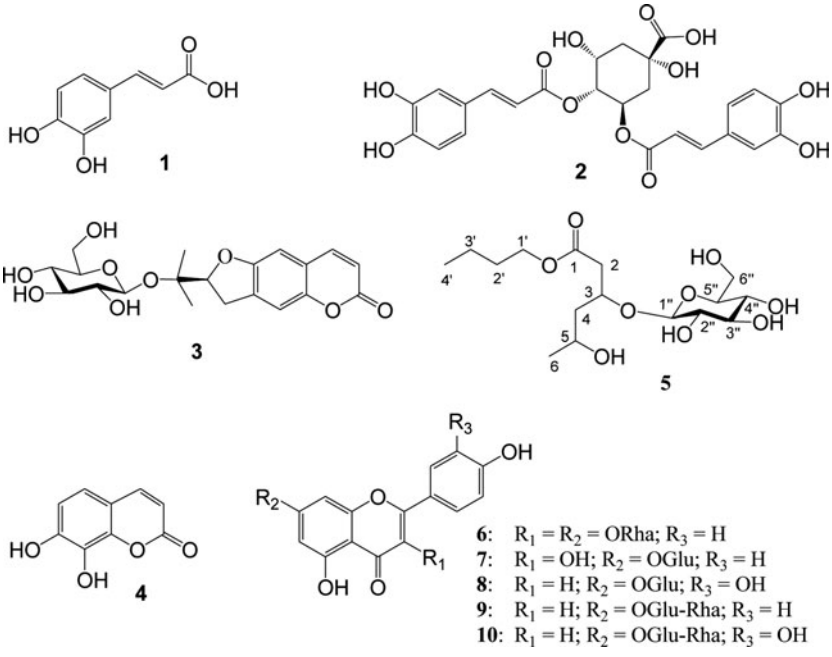

Figure 1. Chemical structures of compounds **1**–**10**.

(3*S*:5*S*)-5-hydroxy-3-[β-D-glucopyranosyloxy]-hexanoic acid butyl ester (**5**), kaempferol 3,7-bismannoside (**6**) (Mulinacci et al. 1995), apigenin 7-*O*-glucoside (**7**), luteolin 7-*O*-glucoside (**8**) (Matlawska & Sikorska 2002), apigenin 7-*O*-rutinoside (**9**) and luteolin 7-*O*-rutinoside (**10**) (She et al. 2004), were isolated and identified. As a new natural product, the ^{1}H and ^{13}C NMR data of compound **5** were reported here for the first time.

Using a modified $ABTS^{\cdot+}$ method, its radical-scavenging potential was shown in Table 1 by the respective IC_{50} values (mM). All compounds displayed high antioxidant activity except compounds **3** and **5**. Especially, compound **2** exhibited stronger antioxidant activity than Trolox.

Table 1. The effect of concentrations of the antioxidant on the inhibition of the ABTS radical.

Number	Compound name	IC_{50} (mM)[a]
1	Caffeic acid	3.89 ± 0.044
2	3,4-Dicaffeoylquinic acid	0.87 ± 0.039
3	Marmesinin	NR
4	7,8-Dihydroxycoumarin	2.57 ± 0.258
5	5-Hydroxy-3-[β-D-glucopyranosyloxy]-hexanoic acid butyl ester	NR
6	Kaempferol 3,7-bismannoside	8.88 ± 0.560
7	Apigenin 7-*O*-glucoside	13.78 ± 0.994
8	Luteolin-7-*O*-glucoside	9.68 ± 0.533
9	Apigenin 7-*O*-rutinoside	4.10 ± 0.621
10	Luteolin-7-*O*-rutinoside	2.62 ± 0.409
11[b]	Trolox	1.33 ± 0.187

Note: NR, not react at the condition described.
[a] The inhibition was recorded at 10 min of reaction (ABTS method) and IC_{50} value was measured using PROBIT model: PROBIT (p) = intercept + BX (Covariates X are transformed using the base 10,000 logarithm). Each value corresponds to the mean and standard deviation of the duplicate of the five concentration.
[b] Position control group.

Previous clinical and experimental studies described that antioxidant supplementation including flavonoids and vitamins could inverse the oxidant-mediated cough depression by modulating the inflammatory process in lung disease (Brozmanova et al. 2006, 2007). In this study, the structures and antioxidant activities of these 10 compounds, in certain level, verified traditional knowledge of the Zhuang people in using *G. piloselloides* as effective antitussive. However, more experiments still need to be conducted to further prove why this plant can be used as spices for wine-brewing and cooking meat.

2.2. *Taxonomy*

The phylogenetic relationship between *Gerbera* and the closely related Asteraceous genus *Piloselloides* has been long disputed and inconsistent. However, the previous studies on *Gerbera* were focused on its morphology and molecular phylogeny (Hansen 1990; Wu & Peng 2002; Baird et al. 2010), and no chemotaxonomic discussion was conducted within the genus *Gerbera*.

In this study, two caffeic acids (**1** and **2**), two coumarin compounds (**3** and **4**) and five flavonoid compounds (**6**–**10**) were found in *G. piloselloides*. Compound **5** was newly found as a natural product. Compounds **1**, **2**, **4**, **6**, **9** and **10** were isolated from the genus *Gerbera* for the first time, and compound **3** had never been found in other *Gerbera* species. The flavonoid compounds (**7**) and (**8**) have been detected previously from *Gerbera jamesonii* (Asen 1984). The co-occurrence of flavonoids (**7** and **8**) and the coumarin (**4**) with its derivatives, such as 3,8-dihydroxy-4-methoxycoumarin, 6-hydroxy-4-methoxy-5-methylcoumarin and 5,8-dihydroxy-7-(4-hydroxy-5-methyl-coumarin-3-) coumarin, in *G. piloselloides* and *G. jamesonii*, indicates that there are similar metabolic pathways in these two species, which partially explained the confusion of morphological taxonomy in two genera, *Gerbera* and *Piloselloides* (Halim et al. 1980; Gu et al. 1989). Meanwhile, the presence of flavonoid glycosides (**6**–**10**) in one species, *G. piloselloides*, suggested that compounds **6**–**10** share similar bio-synthetic pathway in the plant. In the previous study, coumarins are the principle compounds which had been detected from *Gerbera* species (Xiao & Ding 2002). In this study, however, most of compounds which had been isolated were flavonoids. Based upon the above-mentioned comparisons and since there were no reports about the chemical constituents from the genus *Piloselloides*, this study in certain level supported the new classification in the *Flora of China* that *G. piloselloides* belongs to the genus *Piloselloides.* However, more experiments and technologies, such as HPLC-TOF-ESI-MS technique and principal component analysis, are needed to conduct to further define the relationship of taxonomy between *Gerbera* and *Piloselloides*.

3. Conclusions

Using activity-guided isolation method, (3*S*:5*S*)-5-hydroxy-3-[β-D-glucopyranosyloxy]-hexanoic acid butyl ester as a new natural product with nine known compounds were isolated from genus *Gerbera*. The antioxidant assay of compounds partially verified Zhuang people's traditional knowledge that they use this plant as effective antitussive. The results also supported the classification in the *Flora of China* for *G. piloselloides*, which reinstated *Piloselloides* to generic rank.

Supplementary material

Experimental details relating to this article are available online, alongside Figures S1–S5 and Table S1.

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