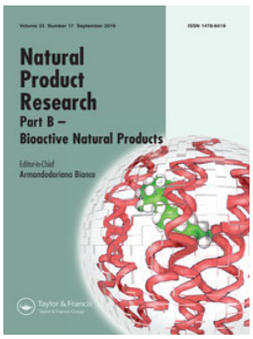

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SHORT COMMUNICATION

Phenolic compounds from the aerial parts of *Clematis viticella* L. and their *in vitro* anti-inflammatory activities*

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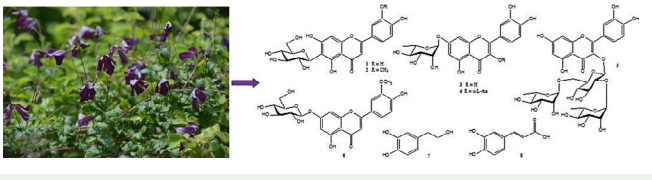
ABSTRACT

Phytochemical investigations on the EtOH extract of *Clematis viticella* led to the isolation of six flavonoid glycosides, isoorientin (**1**), isoorientin 3′-*O*-methyl ether (**2**), quercetin 7-*O*-α-L-rhamnopyranoside (**3**), quercetin 3,7-di-*O*-α-L-rhamnopyranoside (**4**), manghaslin (**5**) and chrysoeriol 7-*O*-β-D-glucopyranoside (**6**), one phenylethanol derivative, hydroxytyrosol (**7**), along with three phenolic acids, caffeic acid (**8**), (*E*)-*p*-coumaric acid (**9**) and *p*-hydroxybenzoic acid (**10**). The structures of the isolates were elucidated on the basis of NMR and HR-MS data. All compounds were isolated from *C. viticella* for the first time. Compounds **7** and **8** showed significant anti-inflammatory activity at 100 µM by reducing the release of NO in LPS-stimulated macrophages comparable to positive control indomethacin. Compounds **3** and **7** exhibited anti-inflammatory activity through lowering the levels of TNF-α while **1**, **3** and **5** decreased the levels of neopterin better than the positive controls.



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1. Introduction

Clematis L., being of the largest genera of the family Ranunculaceae is represented around 300 species worldwide. Several *Clematis* species have extensively been used for the treatment of inflammation-associated diseases, i.e. chronic skin diseases as well as treating wind-cold rheumatism among others in worldwide traditional medicines (Chawla et al. 2012). In Turkish folk medicine also *Clematis* species were reported to be used externally to relieve rheumatic pains (Yesilada and Küpeli 2007). Previous phytochemical studies on the aerial and underground parts of various *Clematis* species have demonstrated that saponins, flavonoids and lignans constitute the major chemical classes of the genus (Chawla et al. 2012; Chang et al. 2017). Several triterpenoid saponins and phenolic glycosides from *Clematis* species were reported to possess cytotoxic, anti-inflammatory and antioxidant activities (Chawla et al. 2012; Chang et al. 2017). In the flora of Turkey, the genus *Clematis* is represented by five species including *C. viticella* (Davis et al. 1965). As no phytochemical and biological activity studies has been performed on this species and the widely utilization of *Clematis* species in different folk medicine prompted us to perform a phytochemical study on this plant. Further, we also aimed to evaluate the effects of the isolates on the release of some inflammatory markers such as NO, TNF-α and neopterin in order to justify potential anti-inflammatory constituents.

2. Results and discussion

Phytochemical studies on the alcoholic extract of *C. viticella* yielded ten phenolic compounds namely isoorientin (**1**), isoorientin 3′-*O*-methyl ether (**2**), quercetin 7-*O*-α-L-rhamnopyranoside (**3**), quercetin 3,7-di-*O*-α-L-rhamnopyranoside (**4**), manghaslin (**5**), chrysoeriol 7-*O*-β-D-glucopyranoside (**6**), hydroxytyrosol (**7**), caffeic acid (**8**) as well as a mixture of (*E*)-*p*-coumaric acid (**9**) and *p*-hydroxybenzoic acid (**10**) (Figure 1). The structural characterization of the compounds were accomplished by 1D and 2D NMR spectra as well as HRESIMS analysis. All compounds are being reported for the first time from *C. viticella*, while **2**, **4**, **5** and **6** are new for the genus *Clematis*.

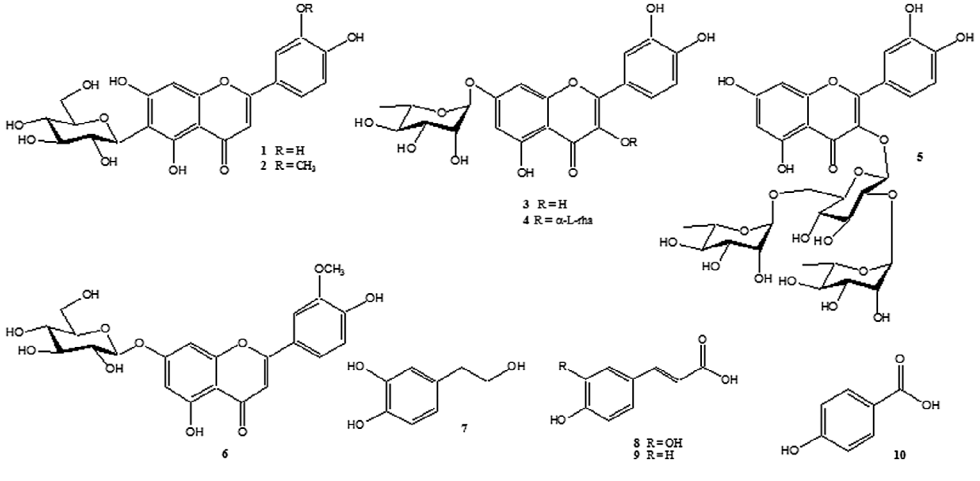

Figure 1. Chemical structures of compounds (**1–10**) isolated from *C. viticella*.

Inflammation is a complex process in which many cytokines, prostaglandins and some proinflammatory mediators are involved including NO, TNF-α. On the other hand, neopterin which is synthesized by activated macrophages, is regarded as a marker of inflammation and of immune system activation. Eventually in some previous studies plant extracts and isolates were evaluated for their effects on neopterin production (Kuehnl et al. 2013; Gostner et al. 2015). The pure compounds isolated from *C. viticella* (**1–8**) were evaluated for their potential anti-inflammatory activities on their inhibitory effects on the release of NO, TNF-α and neopterin (Figure 2). All the compounds were first evaluated for their potential cytotoxic effects on RAW264.7 macrophage cells by using MTT assay. According to the results, compounds were determined as non-toxic (cell viability was over 70%) at 100 µM. Then, the anti-inflammatory activities of the isolates (**1–8**) were evaluated by measuring NO, TNF-α and neopterin levels in supernatants of LPS stimulated macrophage cells at the same concentration. As shown in Figure 2, compounds **7** and **8** exhibited a significant decrease on NO levels, more potent than the positive controls indomethacin and L-NAME, indicating the potential anti-inflammatory activities of both compounds. Regarding the effects of tested compounds on TNF-α levels, as shown in Figure 2 compounds **7** and **3** exerted significant antiinflammatory activity by inhibiting the release of TNF-α which is more potent than the positive control indomethacin. Compounds were also investigated for their effects on neopterin as a further support for the antiinflammatory activity. All tested compounds attenuated the level of neopterin being **1**, **3** and **5** are most active ones. Taken together, particularly, compounds **7** and **3** are the most potential antiinflammatory compounds and merit further detailed *in vitro* and *in vivo* antiinflammatory studies.

Hydroxytyrosol (**7**) is one of the important bioactive metabolite of *Olea europea*. It was reported to possess strong antinflammatory activity by inhibiting the production of NO, PGE_2 (Richard et al. 2011). Our findings are in line with these results of this study. The anti-inflammatory activities of caffeic acid was also reported in previous studies (Yang et al. 2013). Concerning flavonoids, the antiinflammatory activities of some of the flavonoids were reported previously (Lee 2015; Küçükboyacı et al. 2016) in some different test systems. However, the antiinflammatory activities of **2**, **3**, **5** and **6** are being reported for the first time in this study. On the other hand, the effects of all the isolated phenolic compounds on neopterin levels are being investigated within this study for the first time.

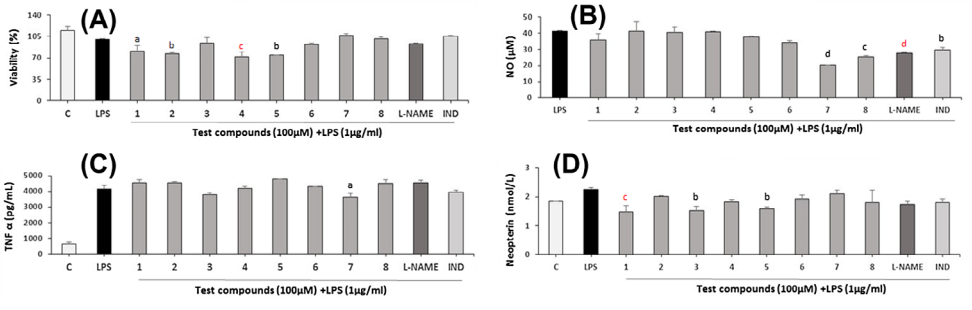

Figure 2. The effects of compounds **1–8** on the viability of RAW264.7 macrophage cells (A), levels of NO (B), TNF-α (C), and neopterin (D).

Notes: Data represent the mean values of three experiments (± SD). The significant differences between groups and their control were defined with a at $p < 0.05$, b at $p < 0.01$, c at $p < 0.001$ and d at $p < 0.0001$. LPS: Lipopolysaccharides from *E. coli*; L-NAME: Nω-Nitro-L-arginine methyl ester hydrochloride; IND: Indomethacin, IND and L-NAME were applied at 100 µM.

3. Conclusion

Ten phenolic compounds were isolated from *C. viticella* for the first time. Compounds **7** and **8** showed significant anti-inflammatory activity at 100 µM by reducing the release of NO in LPS-stimulated macrophages. Compounds **3** and **7** inhibited the production of TNF-α while **1**, **3** and **5** decreased the levels of neopterin significantly.

Disclosure statement

No potential conflict of interest was reported by the authors.

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