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Flavonoid glycosides from Bulgarian endemic *Alchemilla achtarowii* Pawl

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1. Subject and source

The genus *Alchemilla* (Rosaceae) is represented in Bulgarian Flora by 35 species, 12 of which are endemics (Assenov, 1973). The endemic *Alchemilla achtarowii* Pawl. inhabits moist areas near mountain streams over shallow brown forest and rocky mountain-meadow soils of the Stara Planina Mountain between 1700 and 2100 m above the sea level. The species is rare and endangered according to the IUSN criteria and is included in the Red Data Book of R. Bulgaria (Vitkova, in press).

A. achtarowii Pawl. was collected from the Middle Stara Planina Mountain (Bulgaria) in July 2010 and identified by Assoc. Prof. Dr. Antonina Vitkova. Voucher specimen (SOM 165668) was deposited in the Herbarium at the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia.

2. Previous work

Alchemilla species (Lady's mantle, Herba Alchemillae) are used in traditional medicine topically for wounds as well as orally for acute diarrhoea, dysmenorrhoea, menorrhagia, etc. (Bisset, 1994; Ivanov et al., 1977). Different studies showed that the phenolic compounds (tannins, flavonoids, etc.) present in the plant are responsible for the pharmacological activity of Lady's mantle (Jonadet et al., 1986; Lamaison et al., 1991; Filipek, 1992; Schimmer and Lindenbaum, 1995). So far, there are no literature data concerning the chemical composition of the endemic *A. achtarowii*.

3. Present study

Air-dried and powdered aerial parts (70 g) of *A. achtarowii* were extracted with MeOH (3 × 700 ml) at room temperature in an ultrasonic bath for 30 min each. After filtration, the solvent from the combined extracts was evaporated under vacuum to give a total methanolic extract (12.7 g). The latter was further dissolved in distilled water (200 ml) and partitioned

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Table 1
Flavonoid glycosides isolated from *Alchemilla* species.

Species	Type	Compound	Reference
A. coriacea Buser	Qu	Miquelianin (**8**)	Fraisse et al., 1999
A. filicaulis Buser	Qu	Miquelianin (**8**)	Fraisse et al., 1999
A. glabra Neygenf.	Qu	Miquelianin (**8**)	Fraisse et al., 1999
A. mollis (Buser) Rothm.	Qu	Hyperoside (**6**), isoquercetin (**7**), miquelianin (**8**)	Trendafilova et al., 2011
	Ka	*cis*- and *trans*-tiliroside (**1** and **2**)	
	Go	Gossypetin-3-O-β-D-glucopyranosyl-7-O-α-L-rhamnopyranoside (sinocrassoside D_2), gossypetin-3-O-β-D-galactopyranosyl-7-O-α-L-rhamnopyranoside, gossypetin-7-O-α-L-rhamnopyranoside (rhodiolgin)	
A. speciosa Buser	Qu	Hyperoside (**6**), isoquercetin (**7**), quercetin 3-O-l-rhamnoside (quercitrin), miquelianin (**8**), quercetin 3-O-rutinoside (rutin), quercetin 3-O-β-D-sambubioside, quercetin 3-O-β-D-sambubioside-7-O-β-D-glucoside, quercetin 3-O-β-(2"-O-α-L-rhamnopranosyl)-glucopyranoside uronic acid	Felser and Schimmer, 1999
	Ka	Tiliroside (**1/2**), kaempferol 3-O-β-D-glucoside (astragalin, trifolin, **3**), kaempferol 3-O-β-D-glucuronide, kaempferol 3-O-β-(2"-O-α-L-rhamnopranosyl)-glucopyranoside uronic acid	
	Lu	Luteolin 7-O-β-D-glucoside, luteolin 7-O-β-D-rutinoside	
A. vulgaris L.	Qu	Isoquercetin (**7**), rutin, quercetin-3-*O*-α-D-arabinofuranoside (avicularin)	D'Agostino et al., 1998
	Ka	Tiliroside (**1/2**)	
A. xanthochlora Rothm.	Qu	Miquelianin (**8**)	Lamaison et al., 1991; Fraisse et al., 1999
		Gujaverin (**5**)	Fraisse et al., 2000

sequentially with petroleum ether (3 × 100 ml), chloroform (3 × 100 ml) and ethyl acetate (3 × 80 ml) to yield 1.1 g, 0.7 g and 1.2 g of the corresponding extracts, respectively. A portion of the EtOAc extract (0.33 g) was dissolved in MeOH (15 ml) and filtered through celite in order to remove insoluble parts. The clear methanolic solution was concentrated up to 5 ml and applied to a Sephadex LH-20 column (equilibrated with MeOH) to give 4 main fractions [TLC control: Silica gel, EtOAc/MeOH/H_2O, 5:0.8:0.6 and EtOAc/HCOOH/CH_3COOH/H_2O, 100:11:11:26]. The flavonoid containing fraction (50 mg) was further

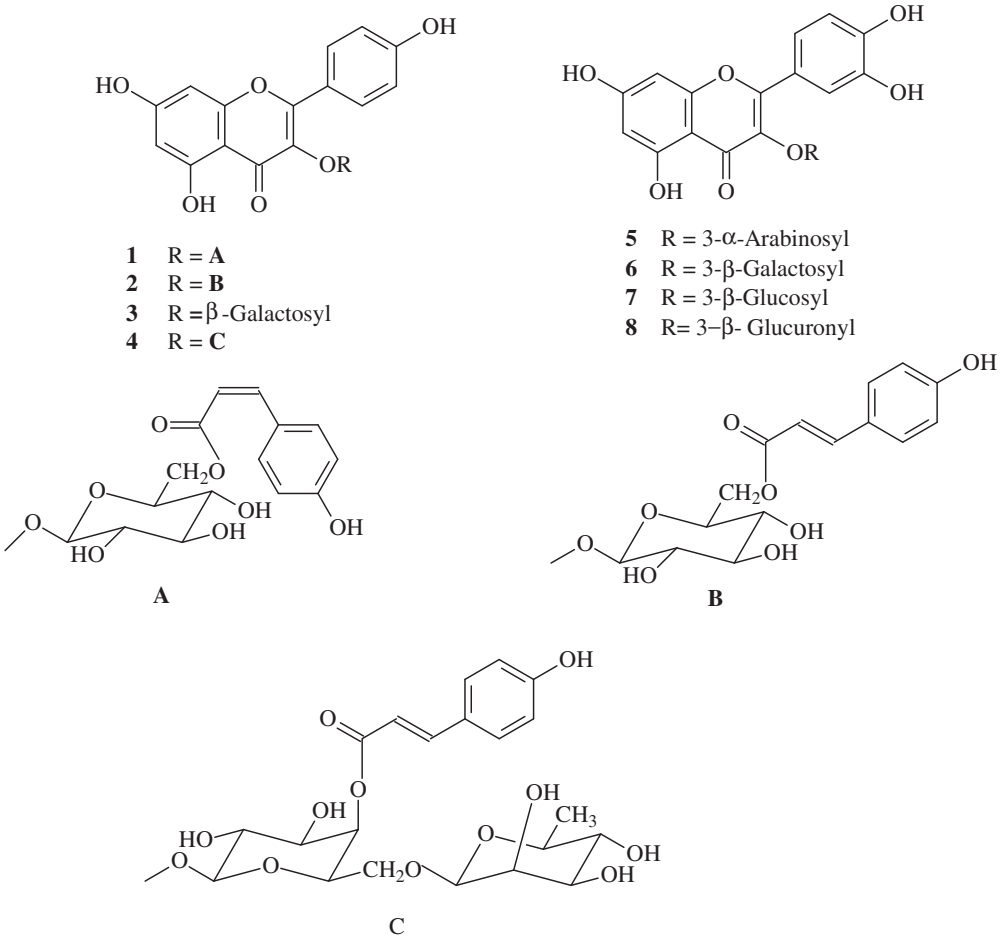


Fig. 1. Structures of flavonoid glycosides **1–8**.

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applied to MPLC on Li Chroprep RP-18 and eluted with increasing concentrations of MeOH in H_2O (20–70%). Repeated MPLC (Li Chroprep RP-18, MeOH/H_2O, 50:50) of selected fractions yielded a mixture of **1** and **2** (7.8 mg), **3** (2.3 mg), **4** (2.6 mg), **5** (7.2 mg), **6** (1.1 mg), **7** (1.2 mg) and **8** (1.9 mg). The isolated compounds (Fig. 1) were identified as kaempferol 3-*O*-β-D-(6″-*Z*-*p*-coumaroyl)-glucopyranoside (*cis*-tiliroside, **1**) (Matlawska et al., 1999), kaempferol 3-*O*-β-D-(6″-*E*-*p*-coumaroyl)-glucopyranoside (*trans*-tiliroside, **2**) (Matlawska et al., 1999), kaempferol 3-*O*-β-D-glucoside (trifolin, **3**) (Jung et al., 2003; He et al., 2010), kaempferol 3-*O*-(4″-*E*-*p*-coumaroyl)-robinobioside (variabiloside G, **4**) (Brasseur and Angenot, 1988), quercetin-3-*O*-α-L-arabinopyranoside (gujaverin, **5**) (Fraisse et al., 2000; Prabu et al., 2006), quercetin-3-*O*-*β*-galactopyranoside (hyperoside, **6**) (Tatsis et al., 2007), quercetin 3-*O*-*β*-glucopyranoside (isoquercetrin, **7**) (Tatsis et al., 2007) and quercetin 3-O-β-D-glucuronide (miquelianin, **8**) (Tatsis et al., 2007) on the basis of their spectral data (UV, ^{1}H NMR, ^{13}C NMR and MS) compared with those published previously and by comparison with authentic samples. It has to be noticed that the isolated amounts do not represent the real concentration of the compounds in the plant.

4. Chemotaxonomic significance

The genus *Alchemilla* (Rosaceae) includes more than 300 species, the majority native to cool temperate and subarctic regions of Europe (Walters and Pawlowski, 1968; Kurtto et al., 2007). The taxonomy of the genus is very complicated, because of the interspecies hybridization and facultative apomixes, causing high morphological variability of the species. *Alchemilla* species as members of Rosaceae family produce flavonoids, phenolic acids and tannins. However, literature survey showed mainly information concerning total flavonoid and tannin content rather than detailed chemical composition. Surprisingly, only few species have been investigated phytochemically so far and flavonoid glycosides isolated from *Alchemilla* species are summarized in Table 1. As can be seen, quercetin and kaempferol glycosides seem to be characteristic for the studied species.

The results described above showed that the EtOAc fraction obtained from the total methanolic extract of *A. achtarowii* contained the same types of flavonoid glycosides – kaempferol (**1**–**4**) and quercetin (**5**–**8**). Among them, **1**, **2**, **6** and **7** have been previously found in *Alchemilla mollis*, *Alchemilla speciosa* and *A. vulgaris*, while trifolin (**3**) and gujaverin (**5**) have been isolated only from *A. speciosa* and *Alchemilla xanthochlora*, respectively. It is noteworthy, that miquelianin (**8**) was detected in all studied species except for *Alchemilla vulgaris*, while compound **4** is reported for the first time in genus *Alchemilla*. Unfortunately, the summarized results are insufficient for any chemotaxonomical conclusions. Moreover, these species are a small part (less than 3%) of the known 300 *Alchemilla* species. Further detailed chemical analyses might be useful in the chemotaxonomy of this genus.

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References

Assenov, I., 1973. Genus *Alchemilla* L.. In: Jordanov, D. (Ed.), 1973. Flora of PR Bulgaria, vol. 5 BAS, Sofia, p. 274.
Bisset, N.G., 1994. Lady's mantle. In: Wichtl, M., Bisset, N.G. (Eds.), Herbal Drugs and Phytopharmaceuticals. Medpharm Scientific Publishers, Stuttgart, p. 52.
Brasseur, T., Angenot, L., 1988. Phytochemistry 27, 1487.
D'Agostino, M., Dini, I., Ramundo, E., Senatore, F., 1998. Phytother. Res. 12, S162.
Felser, C., Schimmer, O., 1999. Planta. Med. 65, 668.
Filipek, J., 1992. Pharmazie 47, 717.
Fraisse, D., Carnat, A., Carnat, A.-P., Lamaison, J.-L., 1999. Ann. Pharm. Fr. 57, 401.
Fraisse, D., Heitzm, A., Carnat, A., Carnat, A.-P., Lamaison, J.-L., 2000. Fitoterapia 71, 463.
He, D., Huang, Y., Ayupbek, A., Gu, D., Yang, Y., Aisa, H.A., Ito, Y., 2010. J. Liq. Chromatogr. Relat. Technol. 33, 615.
Ivanov, I., Landgev, I., Neshev, G., 1977. Bilkite v Bulgaria i izpolzvaneto im (Medicinal plants in Bulgaria and their applications), Zemizdat, Sofia, p. 256.
Jonadet, M., Meunier, M.T., Villie, F., Bastide, J.P., Lamaison, J.L., 1986. J. Pharmacol. 17, 21.
Jung, M.J., Chung, H.Y., Choi, J.H., Choi, J.S., 2003. Phytother. Res. 17, 1064.
Rosaceae (*Alchemilla* and *Aphanes*). In: Kurtto, A., Fröhner, S.E., Lampinen, R. (Eds.), Atlas Florae Europaeae. Distribution of Vascular Plants in Europe, vol. 14. The Committee for Mapping the Flora of Europe & Societas Biologica Fennica Vanamo, Helsinki, p. 200.
Lamaison, J.L., Carnat, A., Petitjean-Freytet, C., Carnat, A.P., 1991. Ann. Pharm. Fr. 49, 186.
Matlawska, I., Sikorska, M., Bylka, W., 1999. Acta. Pol. Pharm. 56, 453.
Prabu, G.R., Gnanamani, A., Sadulla, S., 2006. J. Appl. Microbiol. 101, 487.
Schimmer, O., Lindenbaum, M., 1995. Planta. Med. 61, 141.
Tatsis, E.C., Boeren, S., Exarchou, V., Troganis, A.N., Vervoort, J., Gerothanassis, I.P., 2007. Phytochemistry 68, 383.
Trendafilova, A., Todorova, M., Nikolova, M., Gavrilova, A., Vitkova, A., 2011. Nat. Prod. Commun. 6, 1851.
Vitkova, A. Alchemilla achtarowii Pawl. In: Peev, D. (Ed.), The Red Data Book of R. Bulgaria, vol. 1. Plants, http://e-ecodb.bas.bg/rdb/en/.
Walters, S.M., Pawlowski, B., 1968. *Alchemilla* L. In: Tutin, T.G., Heywood, V.H., Burges, N.A., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.), 1968. Flora Europaea, Rosaceae to Umbelliferae, vol. 2. Cambridge University Press, Cambridge, p. 48.