
Flavonoids from two Turkish Centaurea species and their chemotaxonomic implications

http://researchonline.ljmu.ac.uk/id/eprint/7662/

Article

Citation


LJMU has developed LJMU Research Online for users to access the research output of the University more effectively. Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. Users may download and/or print one copy of any article(s) in LJMU Research Online to facilitate their private study or for non-commercial research. You may not engage in further distribution of the material or use it for any profit-making activities or any commercial gain.

The version presented here may differ from the published version or from the version of the record. Please see the repository URL above for details on accessing the published version and note that access may require a subscription.

For more information please contact researchonline@ljmu.ac.uk

http://researchonline.ljmu.ac.uk/
Flavonoids from two Turkish *Centaurea* species and their chemotaxonomic implications

SHARMEEN UDDIN, LILIAN ALNISOUR, PETER SEGUN, HUSEYN SERVÄ, SEZGIN CELIK, R. SÜLEYMAN GÖKTÜRK, AFAF AL-GROSHI, SHAYMAA AL-MAJMAIE, STEPHANIE T. GUETCHUENG, LUTFUN NAHAR, NICOLA M. DEMPSTER, FYAZ M.D. ISMAIL, KENNETH J. RITCHIE and SATYAJIT D. SARKER

1Mediterranean and Natural Product Research Group, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, James Parsons Building, Byrom Street, Liverpool L3 3AF, United Kingdom
2Altınbas University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Istanbul, Turkey
3Yıldız Technical University, Molecular Biology and Genetics Department, Istanbul, Turkey
4Akdeniz University, Faculty of Science, Department of Biology, 07058, Antalya, Turkey

**ABSTRACT**

*Centaurea asstro-anatolica* Hub.-Mor. and *C. kizildaghensis* Uzunh., E. Doğan & H. Duman, two indigenous perennial herbs from the Turkish flora, belong to the medicinally important genus *Centaurea* L. (fam: Asteraceae), which comprises ca. 600 species worldwide. While various *Centaurea* species are well-known for producing alkaloids, flavonoids, lignans and triterpenoids, there is no report on any thorough phytochemical work on any of these two species available to date. In continuation of our phytochemical and bioactivity studies on the Turkish *Centaurea* species, four flavonoids apigenin (1), apigenin 7,4′-dimethyl ether (2), genkwanin (3) and quercetin (4) were isolated from the methanol extracts of the aerial parts of *C. asstro-anatolica* and *C. kizildaghensis*, for the very first time. The structures of the flavonoids were elucidated conclusively by spectroscopic means, i.e., UV, MS and 1D and 2D NMR data analyses. The distribution of these flavonoids (1-4) within the genus *Centaurea* and their possible chemotaxonomic implications within the genus *Centaurea* or the family Asteraceae have been discussed.

**ARTICLE HISTORY**

Received: 21 October 2017
Revised: 30 October 2017
Accepted: 31 October 2017
ePublished: 11 December 2017

**KEYWORDS**

*Centaurea asstro-anatolica*
*Centaurea kizildaghensis*
*Asteraceae*
*HPLC*
*Flavonoids*
*Chemotaxonomy*

© 2017 Islamic Azad University, Shahrood Branch Press, All rights reserved.

1. Introduction

*Centaurea asstro-anatolica* Hub.-Mor. and *Centaurea kizildaghensis* Uzunh., E. Doğan & H. Duman are two Turkish endemic species of the genus *Centaurea* L., which includes about 600 herbaceous thistle-like flowering plants worldwide, and 182 species of which come from Turkey (Sarker et al., 1997; Russo et al., 2016). *C. asstro-anatolica* is a 30-60 cm tall woody perennial herb, branched above, leaves are tomentose and hairy, has pink flowers, and prefers macchie, under *Pinus brutia* forest and screes, 460 m above the sea level. This species grows only in Antalya and Muğla Provinces in Turkey (Wagenitz, 1975). On the other hand, *C. kizildaghensis* is a perennial herb with a woody rootstock erect stem up to 52 cm long, slightly striate and scarcely tomentose hairs. It has yellow flowers. This plant prefers serpentine rocky slopes and clearings of a *Pinus nigra* from 1700 m to 1800 m above the sea level. This species only grows in Kazı Dağ, close to Derebacik District, in Konya Province in Turkey (Uzunhisarcıklı et al., 2007). While several species of the genus *Centaurea* are well-known for their uses in folklore medicines for the treatment of diabetes, diarrhoea, hypertension, malaria, microbial infections, rheumatism and ulcers (Sarker et al., 1997; Baytop, 1999; Uğur et al., 2009), there is no report on any medicinal uses of *C. asstro-anatolica* or *C. kizildaghensis* in folklore medicines in Antalya, Muğla or Konya provinces. There is only one report on GC-MS based volatile component analysis and antimicrobial activity assessment of *C. asstro-anatolica* (Uğur et al., 2009), and only anthocyanin content was previously detected in *C. kizildaghensis* (Gökbel et al., 2015). As part of our on-going phytochemical and bioactivity
studies on the Turkish Centaurea species (Shoeb et al., 2005, 2007a-e; Granger et al., 2009; Sarker et al., 2005, 2007, 2012), we now report, for the very first time, on the isolation and identification of flavonoids, apigenin (1), apigenin 7,4'-dimethyl ether (2), genkwanin (3) and quercetin (4) from C. austro-anatolica and C. kizildaghensis (see Fig. 1), highlighting possible chemotaxonomic implications of these flavonoids within the genus Centaurea and the family Asteraceae.

![Molecular structures of the characterized flavonoids from C. austro-anatolica and C. kizildaghensis.](image)

**Fig. 1.** Molecular structures of the characterized flavonoids from C. austro-anatolica and C. kizildaghensis.

## 2. Experimental

### 2.1. Chemicals and regions

Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich (Dorset, UK). All solvents for extraction and chromatography were purchased from Fisher Scientific (Loughborough, UK). NMR solvents were from GOSS Scientific (Crewe, UK). Mass spectroscopic analyses were performed on a Finnigan MAT 95 spectrometer. The $^1$H- and $^{13}$C-NMR spectra were recorded at 600 MHz and 150 MHz, respectively, on an Ultrashield Bruker AMX 600 NMR spectrometer. Methyl, methylene and methane carbons were distinguished by DEPT experiments. Homonuclear $^1$H connectivity was determined by using the COSY experiment. $^1$H-$^{13}$C one-bond connectivity was established with HMQC gradient pulse factor selection. Two- and three-bond connectivity was confirmed by HMBC experiments. Chemical shifts are reported in δ (ppm) and coupling constants (J) were measured in Hz.

### 2.2. Plant materials

The flowering aerial parts of C. austro-anatolica Hub.-Mor. were collected from Antalya, Kumluca District (36° 17' 18'"N, 030° 24' 08'"E), about 250 m above the sea level during July 2015, and a voucher specimen (Gokturk 7888) has been deposited in the Herbarium of the Biology Department of Akdeniz University. Similarly, the flowering aerial parts of C. kizildaghensis Uzunh., E. Doğan & Duman were collected from Konya, Derebucak District (37° 21' 01'"N, 031° 40' 48'"E), 1760 m above the sea level during July 2015, and a voucher specimen (Gokturk 7963) has been deposited in the same herbarium.

### 2.3. Extraction and isolation

Air-dried and ground flowering aerial parts of C. austro-anatolica and C. kizildaghensis (400 g each) were separately macerated overnight at room temperature, sequentially, using solvents of increasing polarity, n-hexane, dichloromethane (DCM) and methanol (MeOH) (3 x 1.5 L each). Extracts were evaporated to dryness using a rotary evaporator (max temp 50 °C). The MeOH extraction afforded the maximum amounts of dried extracts from both species (34 g and 51 g from C. kizildaghensis and C. austro-anatolica, respectively, and were subjected to subsequent chromatographic separations using a combination of solid-phase extraction (SPE), analytical HPLC and preparative and/or semi-preparative HPLC on reversed-phase C$_{18}$ stationary phase.

#### 2.3.1. Solid-phase extraction

A portion (2.0 g) of the MeOH extract of C. austro-anatolica and C. kizildaghensis, was individually subjected to SPE fractionation on a Strata (reversed-phase C$_{18}$, 20 g, manufacturer: Phenomenex) pre-packed cartridge, using eluents of decreasing polarity (water-MeOH mixture: 80:20, 50:50, 20:80 and 0:100, 250 mL each) to obtain four fractions (named as SPE fractions 1-4) from each MeOH extract. All fractions were evaporated to dryness using a rotary evaporator (max temp 50 °C).

#### 2.3.2. Analytical HPLC

All SPE fractions (10 mg/mL) were analysed by analytical HPLC using a Dionex Ultimate 3000 UPLC, coupled with an autosampler, degasser and a photodiode array detector, on a Thermo Scientific™ Hypersil GOLD™ C$_{18}$ column (150 mm x 4.6 mm, 5 μm) connected to a guard column, firstly to have an understanding of their chemical profiles (a linear gradient mobile phase: 30-100% MeOH in water in 30 min followed by 100% MeOH for 10 min, 1 mL/min, each solvent contained 0.1% TFA) and also to develop appropriate methods (various gradients of MeOH in water, 1 mL/min) suitable for separation of compounds using a preparative or semi-preparative HPLC. Injection volume was 20 μL. All chromatograms were monitored at four different wavelengths, 220, 250, 280 and 320 nm, and all separated peaks were analysed after each run using the Chromeleon™ 7.2 for UV-Vis data data. Analytical HPLC was also used to check the purity of compounds isolated from the preparative and/or semi-preparative HPLC.
2.3.3. Preparative and/or semi-preparative HPLC

Preparative and/or semi-preparative HPLC separation was performed on an Agilent 1200 preparative HPLC system, coupled with an autosampler, online degasser and photo-diode-array detector. For preparative separation a HiChrom ACE5 C₁₈ pre-column (150 mm, 21.2 mm, 5 μm; HiChrom; flow rate 10 mL/min) and a Luna semi-prep C₁₈ column (150 mm x 10 mm, 5 μm; Phenomenex; flow rate: 2 mL/min) were used.

While the preparative HPLC purification (a linear gradient mobile phase: 30-70% MeOH in water 30 min followed by 100% MeOH for 10 min, 10 mL/min, each solvent contained 0.1% TFA) of the SPE fraction 2 (230 mg) of the MeOH extract of C. austro-anatolica afforded two flavonoids, quercetin (4, tₑ=15.4 min, 10.9 mg) and apigenin (1, tₑ=19.4 min, 7.8 mg), a semi-preparative HPLC purification (a linear gradient mobile phase: 55-90% MeOH in water in 30 min followed by 100% MeOH for 10 min, 2 mL/min, each solvent contained 0.1% TFA) was carried out for the purification of the SPE fraction 3 (28 mg) to obtain genkwanin (3, tₑ=17.2 min, 8.3 mg). Similar preparative HPLC purification (a linear gradient mobile phase: 20-60% MeOH in water in 30 min followed by 100% MeOH for 10 min, 2 mL/min, each solvent contained 0.1% TFA) of the SPE fraction 2 (156 mg) of the MeOH extract of C. kizildaghensis yielded apigenin (1, tₑ=20.2 min, 7.6 mg) and semi-preparative HPLC (a linear gradient mobile phase: 30-90% MeOH in water in 30 min followed by 100% MeOH for 10 min, 2 mL/min, each solvent contained 0.1% TFA) of the SPE fraction 3 (37 mg) afforded apigenin 7,4'-dimethyl ether (2, tₑ=24.6 min, 6.1 mg).

2.3.4. Identification of compounds

All isolated flavonoids were identified conclusively by comprehensive spectroscopic data analysis, e.g., UV, MS and 1D and 2D NMR, and by comparison with respective published data.

**Apigenin (1):** Obtained as yellowish brown amorphous solid. UV λₑₘₐₓ (MeOH) nm: 268 and 336. ¹H NMR (600 MHz, CD₃OD) and ¹³C NMR (150 MHz, CD₃OD); Table 1. ESIMS (+ve ion mode) m/z: 271 [M+H]⁺ corresponding to C₁₈H₁₄O₄ (Mabry et al., 1970; Agrawal, 1989).

**Apigenin 7,4'-dimethyl ether** (also known as genkwanin 4'-methyl ether, 2): Obtained as yellowish brown amorphous solid. UV λₑₘₐₓ (MeOH) nm: 268 and 334. ¹H NMR (600 MHz, CD₃OD) and ¹³C NMR (150 MHz, CD₃OD); Table 1. ESIMS (+ve ion mode) m/z: 299 [M+H]⁺ corresponding to C₁₈H₁₆O₄ (Mabry et al., 1970; Agrawal, 1989).

**Genkwanin (3):** Obtained as yellowish brown amorphous solid. UV λₑₘₐₓ (MeOH) nm: 267 and 333. ¹H NMR (600 MHz, CD₃OD) and ¹³C NMR (150 MHz, CD₃OD); Table 1. ESIMS (-ve ion mode) m/z: 283 [M-1]⁻ corresponding to C₁₈H₁₅O₄ and 567 [2M-1]⁻ (Mabry et al., 1970; Narain, 1976; Agrawal, 1989; Ayatollahi et al., 2009).

**Quercetin (4):** Obtained as yellowish brown amorphous solid. UV λₑₘₐₓ (MeOH) nm: 269 and 352. ¹H NMR (600 MHz, CD₃OD) and ¹³C NMR (150 MHz, CD₃OD); Table 1. ESIMS (-ve ion mode) m/z: 301 [M-1]⁻ corresponding to C₁₉H₁₇O₄ and 603 [2M-1]⁻ (Mabry et al., 1970; Agrawal, 1989).

3. Results and Discussion

3.1. Isolation and identification of flavonoids

A combination of SPE, analytical, semi-preparative and preparative reversed-phase HPLC analyses of the MeOH extracts of C. austro-anatolica and C. kizildaghensis yielded four flavonoids, including three flavones, i.e., apigenin (1), apigenin 7,4'-dimethyl ether (2) and genkwanin (3), and a flavanol, quercetin.

<table>
<thead>
<tr>
<th>Position</th>
<th>¹H NMR chemical shift (in ppm)</th>
<th>¹³C NMR chemical shift (in ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>6.67 (1H, s)</td>
<td>6.40 (1H, s)</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>6.61 (1H, d, J=2.2)</td>
<td>6.28 (1H, d, J=2.3)</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>6.84 (1H, d, J=2.2)</td>
<td>6.45 (1H, d, J=2.3)</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>'1'</td>
<td>-</td>
<td>121.4</td>
</tr>
<tr>
<td>2'</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2' and 6'</td>
<td>7.91 (2H, d, J=8.9)</td>
<td>7.56 (2H, d, J=8.8)</td>
</tr>
<tr>
<td>3'</td>
<td>-</td>
<td>117.2</td>
</tr>
<tr>
<td>4'</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5'</td>
<td>6.97 (2H, d, J=8.9)</td>
<td>6.98 (2H, d, J=8.8)</td>
</tr>
<tr>
<td>6'</td>
<td>-</td>
<td>7.32 (1H, d, J=8.8)</td>
</tr>
<tr>
<td>7-OME</td>
<td>-</td>
<td>3.98 (3H, s)</td>
</tr>
<tr>
<td>4'-OME</td>
<td>-</td>
<td>3.86 (3H, s)</td>
</tr>
</tbody>
</table>
(4) in reasonable yields (6-11 mg). While apigenin (1) was identified from both species, C. austro-anatolica also afforded genkwanin (3) and quercetin (4), and C. kizildaghensis provided apigenin 7,4′-dimethyl ether (2), which is also known as genkwanin 4′-methyl ether. The UV spectra obtained from the HPLC-PDA analysis indicated that these compounds were flavonoids (Mabry et al., 1970). 1H and 13C NMR analyses (Table 1) together with 2D NMR experiments, i.e., COSY, HMBC and HSQC, unequivocally established the structures of these flavonoids. ESI-MS analysis of these flavonoids (1-4) showed respective pseudomolecular ions, either [M+H]+ or [M-H]- ions and thus further confirmed the identity of these flavonoids.

All spectroscopic data were comparable with respective literature data. Although all these flavonoids are known natural products, they have not been previously reported from any of these species. Besides, the occurrence of apigenin 7,4′-dimethyl ether (2) and genkwanin (3) is rather limited within the genus Centaurea.

3.2. Distribution and chemotaxonomic implications

The genus Centaurea is well-known for producing various types of secondary metabolites, mainly, alkaloids, flavonoids, lignans and terpenoids (Sarker et al., 1997). However, all these compounds do not occur in all species of this genus; there are significant variations in the distribution of these compounds within this genus. Flavonoids and their glycosides occur in certain Centaurea species, and can be used as chemotaxonomic markers (De Oliveira et al., 2017). Flavonoids have also been successfully employed as chemotaxonomic markers within the Asteraceae (Emerenciano et al., 2001); at the tribe and sub-tribe levels, because flavonoids possess a wide structural diversity and have been isolated from a number of species of the Asteraceae, and it was previously shown that flavonoids could be used as taxonomic markers at lower hierarchical levels (Crawford, 1978).

Apigenin (1) is a well-known flavone widely distributed in the genus Centaurea, e.g., C. arenaria, C. chilensis, C. cyanus, C. davidovii, C. galicicae, C. jacea, C. macrocephala, C. mircanthos, C. montana, C. nervosa, C. nicaeensis, C. parilica, C. phrygia, C. repens, C. rupestrotitis, C. saderiana, C. scabiosa, C. scoparia, C. schischkinii, C. soskae, C. stenolepis, C. suaveolens, C. tomorosii, C. triunfetti, C. urvillei, and C. virgata (Negrete et al., 1988; Christensen, 1991; Gonnet, 1993; Youssef and Frahm, 1995; Peter and Dosa, 2002; Zheng et al., 2004; Shoeb et al., 2005; Csapi et al., 2010; Gulcemal et al., 2010; Forgo et al., 2012; Hammoud et al., 2012; Pirvu et al., 2012; Csupor et al., 2013; Nikolova and Bancheva, 2013; Tesevic et al., 2014; Mishio et al., 2015; Tuzun et al., 2017). Similarly, quercetin (4) is also quite common in the genus Centaurea, e.g., C. askoyi, C. amaena, C. bracteata, C. chilensis, C. collina, C. cyanus, C. fioccosa, C. horrida, C. isaurica, C. kotschyri, C. macrocephala, C. mircanthos, C. omphalotricha, C. rupestrotitis, C. rupestris, C. scabiosa, C. suaveolens (Kamanzi et al., 1982; Negrete et al., 1987, 1988; Oksuz and Putun, 1987; Peter and Dosa, 2002; Flamini et al., 2000, 2001, 2004; Mouffok et al., 2012; Pirvu et al., 2012; Curkovic-Perica et al., 2014; Mishio et al., 2015; Albayrak et al., 2017). Both apigenin (1) and quercetin (4) are also found in other genera of the family Asteraceae.

The methylated derivatives of apigenin, i.e., apigenin 7,4′-dimethyl ether (2) and genkwanin (apigenin 7-methyl ether, 3) are rather rare flavones. To the best of our knowledge, genkwanin (3) was previously reported from only one Centaurea species, i.e., C. urvillei (Ulbelen and Oksuz, 1982), but the flavone, apigenin 7,4′-dimethyl ether (2), has never been reported from the genus Centaurea. However, genkwanin (3) has been reported from other genera of the family Asteraceae e.g., Artemisia, Baccharis, Chromolaena, Gueldneraestetica and Vernonnia, and its chemotaxonomic value is well-documented (Nakasugi and Komai, 1998; Kraft et al., 2003; Li et al., 2008; Avula et al., 2009; Piao et al., 2012; De Oliveira et al., 2017). Similarly, apigenin 7,4′-dimethyl ether (2) was reported from the genus Calea (C. tenuifolia) of the family Asteraceae (Koehler et al., 2002). Within the genus Centaurea, apigenin-based flavones including their glycosides and glucuronides are quite common, but methylation of the hydroxyls of the apigenin skeleton, as in 2 and 3, may create a sub-chemical group indicating the presence of a slightly more advanced biosynthetic pathway in certain Centaurea species.

4. Concluding remarks

Isolation of the flavones (1-3) and the flavonol (4) from these two relatively less investigated Centaurea species from the Turkish flora, contributes to the understanding of the chemistry of the genus Centaurea, and also identifies new sources for relatively less common flavones, apigenin 7,4′-dimethyl ether (2) and genkwanin (3). The co-occurrence of these flavonoids within the genus Centaurea or the family Asteraceae may be chemotaxonomically important, especially when controversies about phylogeny and affinity of infra-familial groups as well as uncertain positioning of some genera of the family Asteraceae still exist.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgments

EPSRC National Mass Spectroscopy Service (NMSS), Swansea, is thanked for conducting the MS experiments on the isolated compounds.
References


Nikolova, M., Bancheva, S., 2013. Surface flavonoids


