Scientia Pharmaceutica www.scipharm.at

Research article Open Access

Phenolics as Chemosystematic Markers in and for the Genus *Crepis* (Asteraceae, Cichorieae)

Christian ZIDORN *, Birthe SCHUBERT, Hermann STUPPNER

Institut für Pharmazie, Universität Innsbruck, Innrain 52, A-6020 Innsbruck, Austria.

* Corresponding author. Christian.H.Zidorn@uibk.ac.at (C. Zidorn).

Sci Pharm. 2008; 76: 743–750 doi:10.3797/scipharm.0810-25

Published: November 28th 2008 Received: October 28th 2008
Accepted: November 27th 2008

This article is available from: http://dx.doi.org/10.3797/scipharm.0810-25

Abstract

In contrast to the huge variety of sesquiterpene lactones reported, only a limited number of flavonoids and phenolic acids are known from the genus *Crepis*. Compounds detected in the course of this investigation encompass flavonoids luteolin, luteolin 7-*O*-glucoside, luteolin 7-*O*-gentiobioside, luteolin 7-*O*-glucuronide, and luteolin 4'-*O*-glucoside and caffeic acid derivatives chlorogenic acid, 3,5-dicaffeoylquinic acid, caffeoyltartaric acid, and cichoric acid. The spectrum of compounds found in the flowering heads of plants of the genus *Crepis* are useful chemosystematic markers both to differentiate between species and to characterize the genus and help delimiting it from morphologically similar genera within the Cichorieae tribe of the Asteraceae family.

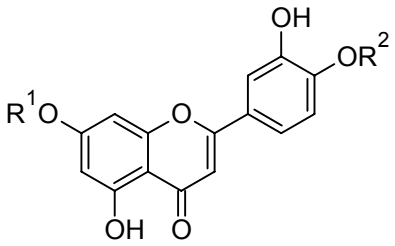
Keywords

Crepis • Asteraceae • Flavonoids • Caffeic Acid Derivatives • Chemosystematics

Introduction

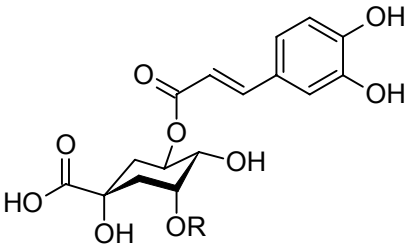
The genus *Crepis* encompasses around 200 species, including about 70 European representatives [1]. In Austria approximately twenty species occur as native elements of the flora [2]. Recent molecular investigations revealed that *Crepis* in its current delimitation is polyphyletic and that in order two obtain a monophyletic genus *Crepis* s.str., the genera *Askellia* (comprising e.g. *C. flexuosa* and *C. nana*) and *Lagoseris* (comprising e.g. *C. pulchra*, *C. praemorsa*, and *C. sancta*) have to be (re-)erected [3]. Numerous *Crepis* taxa

have been investigated for their array of sesquiterpene lactones, which is usually dominated by costus lactone type guaianolides [4].

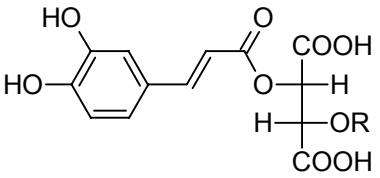


1: $R^1 = R^2 = H$; luteolin
2: R^1 = glucopyranosyl, $R^2 = H$; luteolin 7-*O*-glucoside
3: R^1 = gentiobiosyl, $R^2 = H$; luteolin 7-*O*-gentiobioside
4: R^1 = glucuronyl, $R^2 = H$; luteolin 7-*O*-glucuronide
5: $R^1 = H$, R^2 = glucopyranosyl; luteolin 4'-*O*-glucoside

Fig. 1. Flavonoids detected in flowering heads of the genus *Crepis*.



6: R = H; chlorogenic acid
7: R = caffeoyl; 3,5-dicaffeoylquinic acid



8: R = H; caffeoyltartaric acid
9: R = caffeoyl; cichoric acid

Fig. 2. Caffeic acid derivatives detected in flowering heads of the genus *Crepis*.

Phenolics reported from the genus *Crepis* encompass isoetin from leaves of *C. senecioides* Delile and *C. tectorum* L. [5], chlorogenic acid, 3,5-dicaffeoylquinic acid, caffeoyltartaric acid, cichoric acid, luteolin, luteolin 7-*O*-glucoside, and luteolin 7-*O*-glucuronide from *C. capillaris* (L.) Wallr. flowering heads [6], and apigenin and luteolin from aerial parts of *C. micrantha* Czerep. [7].

In this investigation fifteen *Crepis* species, including two Mediterranean species, were analyzed by HPLC-DAD and HPLC-MS using a chromatographic system already successfully applied to the related genus *Leontodon* [8].

Results and Discussion

HPLC-DAD and HPLC-MS analyses revealed the presence of five luteolin derivatives (**1-5**) (Fig. 1) and four caffeic acid derivatives (**6-9**) (Fig. 2) in the flowering heads of the investigated *Crepis* flowering head samples. As only one sample per species was investigated, the quantification results are given in a semi-quantitative manner [9]. These semi-quantitative results are summarized in Tab. 1. In Tab. 2 (Experimental part) the collection sites of the investigated samples are described. Fig. 3 shows an example of a

chromatogram obtained with the analytical system used for quantifications (see experimental for details).

When comparing the results summarized in Tab. 1 with results obtained in earlier investigation of the genera *Hieracium* [10] and *Leontodon* s.l. [8], which also belong to the Cichorieae (syn.: Lactuceae) subtribe of the Asteraceae family, the array of caffeic acid derivatives of the three genera is of special interest. In *Crepis* and *Leontodon* caffeoyl tartaric acid and cichoric acid occur in most species. In contrast, these two compounds are absent from all species of *Hieracium* s.l. investigated so far. *Crepis* and *Leontodon* are also similar with regards to the spectrum of flowering head flavonoids as taxa of both genera predominantly contain luteolin and a limited number of simple derivatives of luteolin.

From the fifteen *Crepis* taxa investigated only one, *Crepis alpestris*, contains all detected compounds. *Crepis conyzifolia* and *C. tingitana* are characterized by the absence of luteolin 7-*O*-gentiobioside, caffeoyl tartaric acid and cichoric acid.

Tab. 1. Distribution of phenolics in flowering heads of taxa of the genus *Crepis*[a]

Taxon	1	2	3	4	5	6	7	8	9
Crepis alpestris (Jacq.) Tausch	+	+	(+)	(+)	+	++	++	++	++
Crepis aurea (L.) Cass.	+	+	n.d.	+	n.d.	++	+	+	++
Crepis biennis L.	+	+	+	+	n.d.	++	++	++	++
Crepis capillaris (L.) Wallr.	+	+	n.d.	n.d.	n.d.	+	+	++	++
Crepis conyzifolia (Gouan) Kern.	+	++	n.d.	(+)	+	++	++	n.d.	n.d.
Crepis foetida L.	+	+	+	n.d.	n.d.	+	+	+	++
Crepis froelichiana DC.	+	++	n.d.	+	+	++	+	++	+
Crepis jacquinii Tausch subsp. *kerneri* (Rech.f.) Merxm.	+	+	n.d.	n.d.	n.d.	+	+	+	++
Crepis mollis (Jacq.) Asch.	+	+	+	(+)	n.d.	++	++	(+)	++
Crepis nemausensis Gouan	++	++	n.d.	(+)	(+)	++	+	(+)	+
Crepis paludosa (L.) Moench	+	++	n.d.	+	+	+	++	++	++
Crepis pygmaea L.	+	+	+	+	n.d.	+	+	+	++
Crepis rhaetica Hegetschw.	+	+	n.d.	+	n.d.	++	++	++	++
Crepis terglouensis (Hacq.) Kern.	(+)	+	+	+	n.d.	+	+	+	++
Crepis tingitana Ball	+	+	n.d.	(+)	(+)	++	++	n.d.	n.d.

[a] Results of HPLC/DAD investigations; amounts estimated by ratio of peak areas to area of quercetin as internal standard; n.d. = not detectable, (+) = traces i.e. < 0.5 mg/g, + = 0.5 < 5.0 mg/g, ++ > 5.0 mg/g.

Crepis capillaris and *C. jacquinii* subsp. *kerneri* both lack luteolin 7-*O*-gentiobioside, luteolin 7-*O*-glucuronide, and luteolin 4'-*O*-glucoside. *Crepis aurea and C. rhaetica* flowering heads are lacking luteolin 7-*O*-gentiobioside and luteolin 4'-*O*-glucoside. *Crepis biennis*, *C. mollis*, *C. pygmaea*, and *C. terglouensis* contain all investigated compounds except luteolin 4'-*O*-glucoside. Finally, *Crepis froelichiana*, *C. nemausensis*, and *C. paludosa* are characterized by the absence of luteolin 7-*O*-gentiobioside.

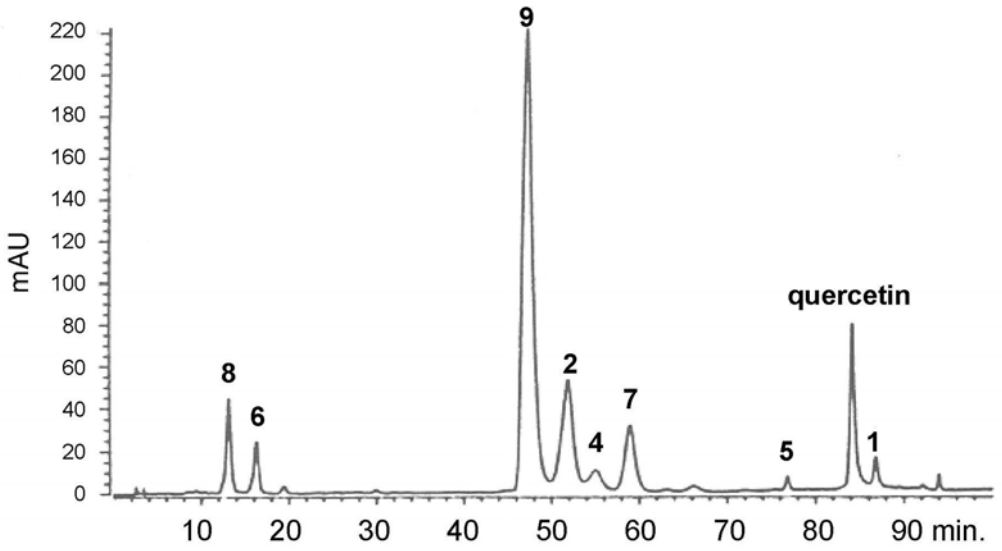


Fig. 3. Chromatogram of an extract of *C. paludosa* L. flowering heads (960822a) obtained with HPLC system 1 at 350 nm (see experimental part for details).

The groupings based on flowering head phenolics are not congruent with the clades found in the recent molecular investigation by Enke and Gemeinholzer [3]. Moreover, the results have to be interpreted with care as they are based on one sample per species only and it is well established that quantitative patterns of flowering heads phenolics in the Cichorieae are influenced by climatic factors of the growing site [6].

Experimental

Plant Material

The origin of the plant material is summarized in Tab. 2. Per population 50 flowering heads were collected and air-dried. Nomenclature is in congruence with the "Katalog der Gefäßpflanzen Südtirols" [11] for plants occurring in Austria and the South Tyrol, follows Flora Europaea for *C. tingitana* [12] and Flora alpina for *C. nemausensis* [synonym: *Crepis sancta* (L.) Babc. subsp. *nemausensis* (Gouan) Babc.] [13]. Voucher specimens of all investigated samples except *C. rhaetica* are deposited in the private herbarium of Christian Zidorn. Vouchers from *C. rhaetica* are kept in the herbarium of the Tiroler Landesmuseum Ferdinandeum in Innsbruck (IBF) and in the herbarium of the Botanical Garden Berlin-Dahlem (B) and doublet vouchers of *C. tingitana* are kept in the herbarium of the Botanical Garden Berlin-Dahlem (B). Scans of the vouchers representing the analyzed collections are included in the on-line version of this paper.

Tab. 2. Collection data of the investigated *Crepis* samples

Taxon	Locality	Latitude	Longitude	Altitude	Date
C. alpestris	N Buchen/T/A	N 47°20'	E 11°07'	1240 m	26.06.1997
C. aurea	Leutasch/T/A	N 47°24'	E 11°11'	1070 m	07.06.1997
C. biennis	between Mühlau and Arzl/T/A	N 47°17'	E 11°25'	620 m	10.05.1997
C. capillaris	Arzl/T/A	N 47°16'	E 11°25'	600 m	24.06.1997
C. conyzifolia	Heiligwasser/T/A	N 47°13'	E 11°26'	1100 m	20.06.1997
C. foetida	Bhf. Branzoll/TN/I	N 46°24'	E 11°19'	260 m	26.07.2001
C. froelichiana subsp. *froel.*	Armentara/TN/I	N 46°37'	E 11°55'	1880 m	22.06.2001
C. jacquinii subsp. *kerneri*	Latemar/TN/I	N 46°23'	E 11°35'	1850 m	13.07.2003
C. mollis	Leutasch/T/A	N 47°24'	E 11°11'	1070 m	07.06.1997
C. nemausensis	NE Guiglia/EMR/I	N 44°27'	E 10°59'	150 m	05.04.1996
C. paludosa	Püngelbachtal/NW/D	N 50°31'	E 6°20'	580 m	14.07.1996
C. pygmaea	Corno Grande/ABR/I	N 42°27'	E 13°33'	2290 m	16.07.2001
C. rhaetica [14]	Zeblasjoch/T/A	N 46°56'	E 10°18'	2680 m	02.08.1998
C. terglouensis	Albula-Paß/GR/CH	N 46°35'	E 9°50'	2350 m	15.08.1997
C. tingitana	N Casares/AND/E	N 36°28'	W 5°15'	580 m	27.04.1997

Extraction of the plant material.

500.0 mg of dry plant material was mixed with 10.0 ml of a stock solution of quercetin-dihydrate [25.0 mg quercetin-dihydrate (Sigma, St Louis, USA) / 100 ml $CH_3OH/(CH_3)_2CO/H_2O$ (3/1/1)], then 15 ml of a mixture of $CH_3OH/(CH_3)_2CO/H_2O$ (3/1/1) was added and extracted for 5 min with an Ultra-Turrax apparatus at 24000 rounds/min and subsequently filtered; afterwards the plant material was again extracted for 5 min at 24000 rounds/min with 25 ml of a mixture of $CH_3OH/(CH_3)_2CO/H_2O$ (3/1/1). Then the plant material was extracted for 5 min at 24000 rounds/min with a mixture of CH_3OH/H_2O (1/1); the combined extracts were diluted to 100.0 ml with $CH_3OH/(CH_3)_2CO/H_2O$ (3/1/1). Finally 10.0 ml of this solution was brought to dryness in vacuo, re-dissolved in 2.00 ml of $CH_3OH/(CH_3)_2CO/H_2O$ (3/1/1), filtered and used for HPLC analysis.

HPLC analyses

Flavonoids and phenolic acids were identified as reported before [8]. In crude extracts, phenolic acids and flavonoids were identified by their HPLC retention times, HPLC-MS and HPLC-DAD spectra in comparison with authentic reference substances. Amounts of the detected compounds in the investigated flowering heads were estimated by the ratio of the respective peak areas to the area of the internal standard quercetin. HPLC analyses for quantification were performed in triplicate. HPLC system 1 used for the HPLC/UV quantification of phenolic compounds; column: HP-ODS-Hypersil 2.1 mm x 200 mm, with 5 µm particle size (Hewlett Packard, Waldbronn, Germany); guard column: LiChroCart 4 x 4 mm packed with LiChrospher RP-18 material (5 µm particle size) (Merck, Darmstadt, Germany); detection wave length: 350 nm; injection volume: 5.0 µl; mobile phase A: 0.1 % trifluoroacetic acid and 0.5 % tetrahydrofurane in water; mobile phase B: 0.1 %

trifluoroacetic acid and 0.5 % tetrahydrofurane in CH_3OH; flow rate: 0.220 ml/min; linear gradient: 0 min 85 % A, 15 % B; 25 min 72.5 % A, 27.5 % B; 55 min 69.5 % A, 30.5 % B; 65 min 67.5 % A, 32.5 % B; 95 min 35 % A, 65 % B; stop time: 100 min; post time: 15 min. Retention times [in chronological order (min)]: **8**, 12.9; **6**, 16.9; **3**, 40.1; **9**, 48.9; **2**, 54.1; **4**, 57.9; **7**, 60.3; **5**, 76.5; quercetin (internal standard), 84.2; and **1**, 87.3.

HPLC system 2 used for the HPLC/MS identification of phenolic compounds; column: LiChrospher RP-18 4 mm x 125 mm, with 5 µm particle size (Merck, Darmstadt, Germany); guard column: LiChroCart 4 x 4 mm packed with LiChrospher RP-18 material (5 µm particle size) (Merck, Darmstadt, Germany); detection wave length: 350 nm; injection volume: 10.0 µl; mobile phase A: 0.3 % acetic acid in water; mobile phase B: CH_3OH; mobile phase C: CH_3CN; flow rate: 1.00 ml/min; linear gradient: 0 min 85 % A, 15 % B, 0 % C; 5 min 75 % A, 15 % B, 10 % C; 25 min 60 % A, 15 % B, 25 % C; 35 min 55 % A, 15 % B, 30 % C; 40 min 15 % A, 15 % B, 70 % C; stop time: 45 min; post time: 10 min. Retention times (min): **8**, 4.5; **6**, 7.1; **9**, 9.5; **3**, 11.5; **4**, 13.8; **2**, 14.2; **7**, 15.1; **5**, 17.7; quercetin (internal standard), 24.1; and **1**, 24.7.

Acknowledgement

The authors thank Barbara Mayer (Innsbruck) for technical assistance.

Supporting Information

17 coloured vouchers of *Crepis* species are available in the online version (Format: PDF, Seize: 8.34 MB): http://dx.doi.org/10.3797/scipharm.0810-25.

Authors' Statement

Competing Interests

The authors declare no conflict of interest.

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Phenolische Inhaltsstoffe als chemosystematische Marker innerhalb der und für die Gattung *Crepis* (Asteraceae, Cichorieae)

Zusammenfassung

Im Gegensatz zur großen Vielfalt an Sesquiterpenlaktonen, die aus Taxa der Gattung *Crepis* isoliert wurden, gibt es nur eine begrenzte Anzahl an phenolischen Inhaltsstoffen, die für diese Gattung berichtet wurden. Im Rahmen der vorliegenden Untersuchung wurden die Flavonoide Luteolin, Luteolin-7-*O*-glucosid, Luteolin-7-*O*-gentiobiosid, Luteolin-7-*O*-glucuronid und Luteolin-4'-*O*-glucosid sowie die Kaffeesäurederivate Chlorogensäure, 3,5-Dicaffeoylchinasäure, Caffeoylweinsäure und Cichoriumsäure nachgewiesen. Das in Blütenköpfen von Taxa der Gattung *Crepis* enthaltene Muster an phenolischen Inhaltsstoffen ist sowohl für die Charakterisierung einzelner Arten innerhalb der Gattung als auch für die Abgrenzung der Gattung von morphologisch ähnlichen anderen Gattungen der Tribus Cichorieae verwendbar.