Editorial Manager(tm) for Phytochemistry Reviews
Manuscript Draft

Manuscript Number:

Title: Phytochemistry of Heather (Calluna vulgaris (L.) Hull.) and its altitudinal alteration

Article Type: Special Issue: Plants from High Altitude

Keywords: flavonoids; calluna vulgaris; phenolic compounds; altitude; radical scavengers

Corresponding Author: Dr Franz Bucar,

Corresponding Author's Institution:

First Author: Marlene Monschein

Order of Authors: Marlene Monschein; Jacobo Iglesias Neira; Olaf Kunert; Franz Bucar

Phytochemistry of Heather (*Calluna vulgaris* (L.) Hull.) and its altitudinal alteration

M. Monschein[1], J. Iglesias Neira[2], O. Kunert[3] & F. Bucar[1,*]

[1] Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz, Universitätsplatz 4/1, A-8010 Graz, Austria

[2] Instituto de Investigaciones Marinas (CSIC), Av. Eduardo Cabello 6, E-36208 Vigo, Spain

[3] Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, University of Graz, Universitätsplatz 1, A-8010 Graz, Austria

*Correspondence: Ao. Univ.-Prof. Dr. Franz Bucar, Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz, Universitätsplatz 4/1, A-8010 Graz, Austria

Tel. +43 (0)316 380 5531

Fax +43 (0)316 380 9860

e-mail: franz.bucar@uni-graz.at

Abstract

Calluna vulgaris (L.) Hull. (heather) is the only species within the monotypic genus *Calluna* (Ericaceae). It is a dominant species of heather communities and can be found in most parts of Europe and Northern America from lowland up to alpine regions. Common heather is traditionally used to treat urinary tract disturbances and inflammatory related disorders.

This review covers the current knowledge on phytochemical investigations of *C. vulgaris* which revealed a complex pattern of flavonoid glycosides including acetylated compounds as well as other classes of phenolics (chromones, procyanidins, simple phenols, etc.) Recently, an acetophenone (rodiolinozid) could be identified.

Its occurance over a wide range of altitudinal zones makes *C. vulgaris* an interesting species to study the variation of its metabolic profiles in wild populations growing under different climatic conditions. Within phenolic compounds flavonols showed significant differences in samples collected at different altitudes with increased levels of quercetin glycosides at higher altitudes whereas no significant correlation could be found for caffeoyl quinic acids and the dihydroflavonol glycoside callunin.

Broadening the scope of such investigations by looking at different species and different geographical areas should give a more concrete picture which phenolic compounds, if any, are suitable markers to observe adaptive processes in high altitude plants. Furthermore, investigations on the specific patterns of phenolics at cellular and subcellular level and their variation due to factors like enhanced solar radiation and low temperature should be expanded.

Key words:

Flavonoids, *Calluna vulgaris*, phenolic compounds, altitude, radical scavengers

Introduction

The genus *Calluna* (Ericaceae) is monotypic and contains only one species, *Calluna vulgaris* (L.) Hull. (heather, common heather, ling). It is a small evergreen shrub, native to Europe but introduced also to altantic Northern America and to New Zealand. It is a dominating species in heathland communities in Northwest Europe but can be found from Spain to Scandinavia and from the Azores to the Ural Mountains (Tutin et al., 1972). Like many Ericaceae, it shows specific mycorrhizal infections which primarily enhance nitrogen uptake but also have been shown to permit growth on soils contaminated with heavy metals (Bradley et al., 1981).

Traditionally, flowers and herbs are used to treat urinary tract disorders, and as an antiseptic, woundhealing, antirheumatic, expectorant and choleretic remedy (Kraus et al., 2007; Kumarasamy et al., 2002). Pharmacological studies showed anti-inflammatory (Tunon et al., 1995), antioxidant (Calliste et al., 2001; Deliorman-Orhan et al., 2009; Kaehkoenen et al., 1999), antiproliferative (Calliste et al., 2001) and MAO-A inhibitory effects (Saaby et al., 2009). Its seeds showed moderate antibacterial activity (Kumarasamy et al., 2002). Herbs and flowers of *C. vulgaris* are still widely collected from the wild. Their high content of phenolics (Jalal et al., 1982) and the biological activity of these compounds serves as the basis for its beneficial effect as a medicinal plant.

Variation of secondary metabolite profiles in plants and their regulatory mechanisms has gained increasing interest during the last years. The occurance of *C. vulgaris* over a wide range of altitudinal zones makes *C. vulgaris* a preferable species to study the variation of its metabolic profiles in wild populations growing under different climatic conditions. This review will cover the phytochemical research that has been carried out on this species and will present results from a project which followed up the altitunal variation of flavonoids and phenolic acids over four vegetation periods with the aim to identify possible marker compounds.

***Phytochemical survey of* C. vulgaris**

Herbs of *C. vulgaris* have been intensively studied concerning phenolic compounds which resulted in a number of flavonoids, chromones, procyanidins, phenolic acids, phenols, many of them occur as glycosides. Other compound classes include sterols, triterpenes, common fatty acids as well as ascorbic acid. The information on the phytochemistry of the roots is comparably small and revealed mainly catechins and procyanidins (Jalal et al., 1982). A detailed summary of available data is presented in Table 1, for structures see Figure 1.

Table 1, Figure 1

C. vulgaris follows the regular observation in Ericaceae that flavonol galactosides and arabinosides occur together (Jalal et al., 1982). A complex pattern of acetylated flavonol glycosides is another feature of *C. vulgaris* aerial parts. During the fractionation of a methanolic extract of the dried herbal parts of a commercial sample of *C. vulgaris* herbs by open column chromatography on Sephadex LH-20 and a close examination of the fractions by LC-ESI-MS and NMR of selected isolated compounds we could find, in addition to previously known substances, an acetophenone (**62**), p-coumaroylquinic acid (**52**), tiliroside (**13**) and further acetylated kaempferol and quercetin glycosides (Monschein et al., 2009; Monschein et al., 2008). The rare acetophenone 1-[4-(β-D-glucopyranosyloxy)-2-hydroxy-6-methoxyphenyl]-ethanone (rodiolinozide, annphenone, **62**) could be identified by its NMR data (see Table 2) and was previously only known in *Monochaetum multiflorum*, *Gnaphalium multiceps*, *Prunus armeniaca* as well as *Rhodiola* and *Artemisia* sps. (Satsyperova et al., 1995; Singh et al., 1997).

Table 2

Obviously *C. vulgaris* is of major importance in floral "heather" honeys. Phenylacetic acid, dehydrovomifoliol and 4-(3-oxo-1-butynyl)-3,5,5-trimethylcylohex-2-en-1-one could be identified as markers for *C. vulgaris* honeys (Guyot et al., 1998). Phenolic acids in heather

honey (without definite origin from *Erica* or *Calluna*) recently were analysed by (Dimitrova et al., 2006).

Altitudinal variation of phenolic compound profiles

General aspects

Do environmental factors in different altitudinal zones influence the content of certain phenolic, antioxidative compounds in plants? Several studies have tried to answer this question and in the following we will summarize results from our recent research as well as those from other groups. In our project we performed a comparative study and collected plant material during four growing periods to examine the consistency of the metabolite profiles. Aside from the basic questions to identify marker compounds and follow up their biosynthetic and metabolic pathways, studies like this have another aspect. For plant derived products based on wild-grown plants, environmental and climatic parameters have higher impact on the composition and quantity of secondary metabolites than in those derived from cultivation, where at least some of these factors can be controlled by agricultural techniques. Hence, the generation of data on the variation of the amounts of beneficial secondary metabolites (Boehm et al., 1998; Hooper et al., 2006) is crucial and contributes to the quality parameters of the plant material used.

C. vulgaris offers due to its vertical spread from coastal areas at sea level to alpine regions of about 2700 m above sea level the possibility to perform comparative studies on samples collected from wild populations at different altitudes even within a restricted area. Although at a first glance this seems to be a straightforward approach there are several limitations. Aside from general features of high mountain climate like low temperatures and enormous temperature extremes, heavy winds, high impact of solar radiation, microclimates can change significantly according to sun and wind exposure within short distances of collection sites (Körner 2003). Differences in soil which might influence the qualitative and quantitative composition of phenolics could be of relevance too. However, *C. vulgaris* generally can be

regarded as indicative for acidic and infertile soils exposed to full sun light or moderate shade. Another item which has to be considered is the fluctuation of phenolics within a day (Veit et al., 1996) and within a vegetation period. A detailed study in this respect has been performed by (Jalal et al., 1982) who found in fresh shoots of *C. vulgaris* a considerable variation with the lowest number of phenolics in January and February which was dominated by chlorogenic acid (**49**) and quercetin glycosides. Until May and June the number and amount of catechins and procyanidins, dihydroflavonols, kaempferol and quercetin glycosides increased and stayed more or less constant till December. Some glycosides like arbutin (**61**) and callunin (**35**) showed occasional disappearance. Consequently, sample collection at distinct points during the growing period, e.g. at the flowering stage, is crucial in order to obtain comparable results.

The amount of polyphenol compounds in plant tissues vary apart from genetic factors or age of the plant according to numerous exogenous factors like environmental parameters, time of harvest, infestation with microorganisms and damage caused by different pests including competition with other individuals or species, some of these environmental factors depend on altitude (Blumthaler et al., 1997; Körner, 2003; Seigler, 1998)

Investigations on C. vulgaris *and comparison with related work*

For our investigations, we selected two areas in Styria (Austria) as they offered comparable general climatic conditions and herbal parts of *C. vulgaris* could be sampled at flowering stage at 800m, 1000m and 1500 m above sea level (Naturpark Sölktäler in 2004 and 2005; Koralpe in 2006 and 2007). Additional samples at high altitude (1500m and 1800/1900 m, resp.) were collected in Styria and Carinthia (Austria). Altogether, more than 140 samples of *C. vulgaris* were analysed. Extracts (80 % methanol) from the air dried plant material for qualitative and quantitative analysis were prepared under controlled conditions using an accelerated solvent extraction procedure (Rieger et al., 2008). For quantification, extracts

were separated by a RP-HPLC method as described previously (Rieger et al., 2008) and with modification by (Monschein, 2009).

Results for major phenolic compounds in herbs of heather are summarized in Table 3.

Table 3

Within one altitudinal zone the variation from one year to another was relatively small and the mean values over year 1 – 4 did not differ more than about 15 % for chlorogenic acid (**49**), callunin (**35**), hyperoside (**25**) and isoquercitrin (**26**), whereas kaempferol-3-O-galactoside (**14**) showed a higher degree of variation (up to 30 %). Concerning flavonols, our extracts of *C. vulgaris* yielded significantly greater amounts of **25** with increasing altitude in all vegetation periods. This was also true for **26** with the exception that the amounts at 800 m and 1000 m were not significantly different. The content of **35** did not differ significantly, this dihydroflavonol has a weaker radical scavenging activity compared to quercetin glycosides (DPPH assay, data not reported). The samples collected at two other places in Styria and Carinthia showed very similar results with even higher amounts of **25** and **26** in samples collected at higher altitude (data not shown). So the elevated levels of quercetin glycosides in high altitude samples of *C. vulgaris* seems to be consistent.

The same trend was also observed with other flavonoids: there was a clear indication that flavonols with structural features of effective antioxidants (Pietta, 2000; Rice-Evans et al., 1996) can be found in higher amounts in samples from higher altitude. However, we could not confirm the assumption, that only the content of flavonol-3-O-glycosides with adjacent hydroxyl group in ring B inreases linked to increasing altitude as also for **14** a similar trend as for **26** was observed.

We observed a high variation of the content of **49** also within samples at one altitudinal level which made it impossible to deduce a correlation between the altitude of their collection site and the amount of substance in the extracts. Similar results were obtained for **50** which was quantified in samples of year 3 and 4 (Monschein, 2009; Monschein et al., 2009; Rieger et al.,

2008). On the other hand, Ganzera et al. (2008) found significant increases of caffeic acid derivatives including **49** in flower heads of *Matricaria chamomilla* cultivated between 1000 and 2000 m above sea level.

Figure 2

Figure 2 shows the percentage distribution of phenolic compounds in the samples collected in year 4 and reveals that the relative proportion of **35** and **49** decreased in relation to quercetin glycosides like **25**, **26** and guajaverin (**23**) if samples from 800 m and 1500 m are compared. Samples from year 3 gave a similar picture.

Several experimental data support that enhanced solar, respectively UV-B radiation is correlated to accumulation of highly antioxidative flavonoids (Jaakola et al., 2004; Markham et al., 1998a; Spitaler et al., 2006). In maize leaves of cultivars from high altitudes UV-B radiation induced the biosynthesis of two C-glycosylflavones, maysin and rhamnosylisoorientin, which seemed to be controlled by a *p* homologous transcription factor (Casati et al., 2005). It is interesting to note that flavonoids (above all flavonol and flavone glycosides) have been found associated with chloroplasts (Saunders et al., 1976) and there are indications of their biosynthesis in these plastids (Zaprometov et al., 2003). In a recent review by (Hernandez et al., 2009), the authors concluded that within flavonoids, flavonols are the most probable candidates as antioxidants which act *in vivo* in plants, however, up to now no definite spatio-temporal correlation between flavonoids, their oxidation products and oxidative stress could be confirmed. The preferable role of flavonols over anthocyanidins was supported by our results of investigations of bilberries and elderberries which revealed a negative correlation with altitude for anthocyanidine glycosides (bilberries and elderberries) but increased levels of rutin (quercetin-3-O-rutinoside) in elderberries (Rieger et al., 2008).

Certainly, in addition to flavonoids, carotenes and waxes, antioxidative enzymes (e.g. GSH, glutathionreductase) and ascorbate or tocopherols contribute to the protective effect against oxidative stress (Abdul Jaleel et al., 2009; Mittler, 2002)

Other factors like temperature might have been underestimated which is supported by the observation that low temperature stress influences the morphological changes of plants at high altitudes (Körner, 2003) as well as results from climate chamber experiments performed in the group of C. Zidorn (Albert et al., 2009) who identified low temperature as a key factor for accumalution of phenolic compounds in alpine plants.

Conclusions and perspectives

Phytochemical investigations of *C. vulgaris* mainly covered phenolic compounds which also have been shown to exert significant biological activities regarding inflammatory processes. Other compound classes still lack detailed analyses. Due to its widespread occurance on specific soils from lowland to alpine regions, *C. vulgaris* was investigated for the altitudinal alteration of flavonoids and other phenolics. The study revealed an increased yield of flavonoids particularly of flavonol-3-O-glycosides linked to increasing altitudes. It could be demonstrated that contents of quercetin glycosides generally increased significantly with the altitude of the collection site. For caffeoyl quinic acids (**49**, **50**) and the dihydroflavonol **35** due to high variation within one altitudinal zone no definite correlation could be found.

Broadening the scope of such investigations by looking at different species and different geographical areas should give a more concrete picture which phenolic compounds, if any, are suitable markers to observe adaptive processes in high altitude plants. It would also be interesting to look at the specific patterns at tissue, cellular and subcellular level and their variation, flavonoids associated with chloroplasts would be an interesting target in this respect. Tissue specific analyses should be performed to clarify if a significant correlation mainly can be found for chloroplast containing parts of the plant organism (green leaves, pedicel, calyx) which face an enhanced production of reactive oxygen species (ROS) caused by the photosynthesis process.

Aside from studies of wild populations reflecting the real life situation, growing experiments with genetically uniform plant material in standardised soils at different altitudes as well as climate chamber experiments should be intensified in order to find the relevant exogenous factors which lead to the observed changes in metabolic profiles of high altitude plants.

Acknowledgements

We are grateful for financial support by the Faculty of Natural Sciences, University of Graz, and the Dr.-Heinrich-Jörg and the Gandolph-Doelter foundations. We thank the board of Naturpark Sölktäler for permission to collect plant material. Prof. Peter Dittrich, University of Graz, is acknowledged for advise with statistical data analysis and Dr. Antje Hüfner, University of Graz, for recording NMR spectra of callunin.

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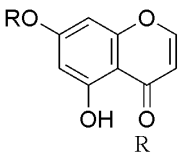
Legends to figures

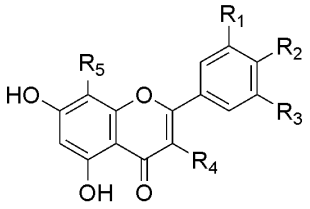
Fig. 1 Selected structures of compounds identified in *Calluna vulgaris*

Fig. 2 Percentage distribution[a] of phenolic compounds in herbs of *Calluna vulgaris* in samples of one collection year

[a] calculated on molar basis (100% = total content of respective compounds in samples of an altitude); 800 m: n=10; 1000 m, 1500 m: n=12; 1500m_1: n=11; samples of 1500_1 were obtained from Zirbitzkogel (Styria), all other samples from Koralpe in year 4 (2008)

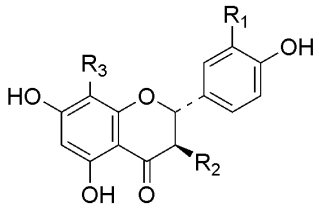


	R
5,7- Dihydroxy-chromone (**1**)	H
5,7- Dihydroxy-chromone (**2**)	*β*-D-glucoside



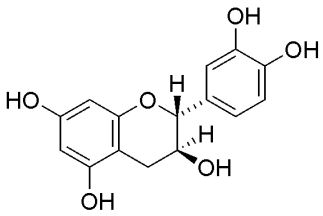
Flavones, flavonols

	R_1	R_2	R_3	R_4	R_5
Apigenin	H	OH	H	H	H
Herbacetin (**5**)	H	OH	H	OH	OH
Isorhamnetin	OCH_3	OH	H	OH	H
Kaempferol (**6**)	H	OH	H	OH	H
Myricetin (**7**)	OH	OH	OH	OH	H
Quercetin (**8**)	OH	OH	H	OH	H

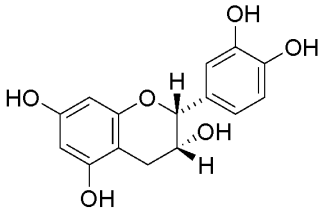


Dihydroflavonols

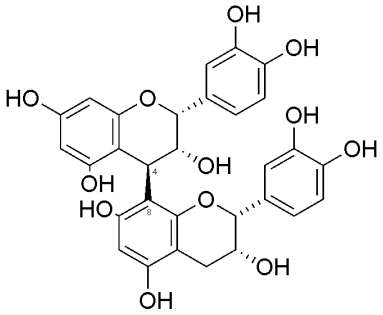
	R_1	R_2	R_3
Taxifolin-3-*O*-glucoside (**34**)	glucoside	OH	H
Callunin (**35**)	H	OH	glucoside
3-Deoxy-callunin (**36**)	H	H	glucoside



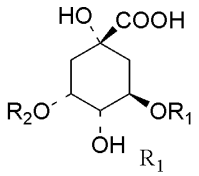
(+)-Catechin (**37**)



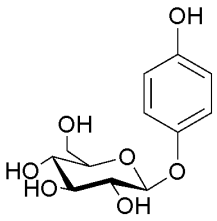
(-)-Epicatechin (**38**)



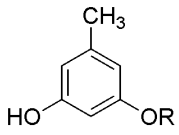
Procyanidin B_2 (**41**)



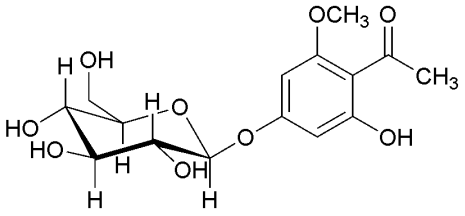
	R_1	R_2
3-*O*-Caffeoyl-quinic acid (**50**)	caffeoyl	H
5-*O*-Caffeoyl-quinic acid (**49**)	H	caffeoyl



Arbutin (**61**)



	R
Orcinol (**59**)	H
Orcinol-β-D-glucoside (**63**)	*β*-D-glucoside



Rodiolinozide (**62**)

Fig. 1 Selected structures of compounds identified in *Calluna vulgaris*

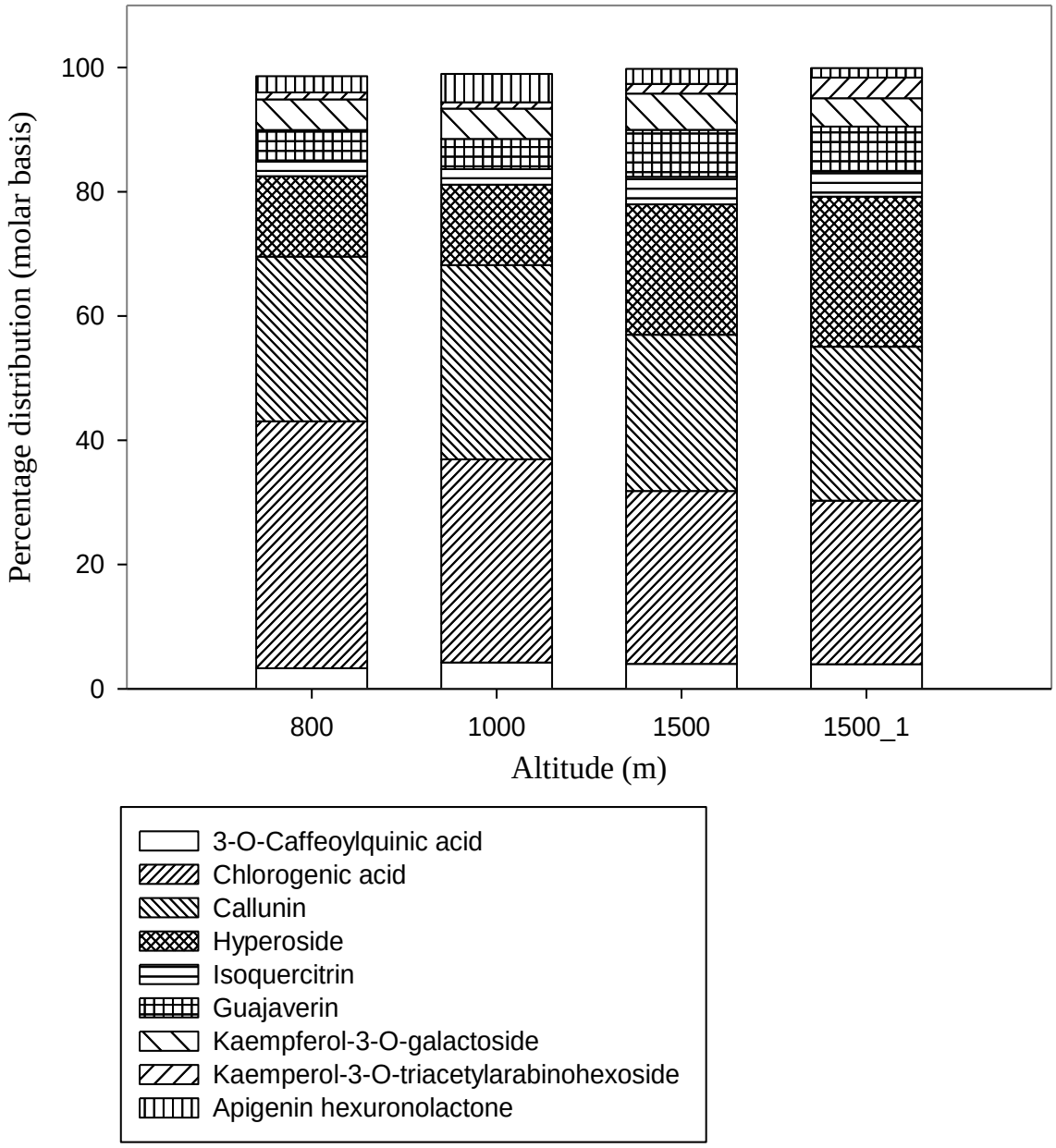


Fig. 2 Percentage distribution[a] of phenolic compounds in herbs of *Calluna vulgaris* in samples of one collection year

[a] calculated on molar basis (100% = total content of respective compounds in samples of an altitude); 800 m: n=10; 1000 m, 1500 m: n=12; 1500m_1: n=11; samples of 1500_1 were obtained from Zirbitzkogel (Styria), all other samples from Koralpe in year 4 (2008)

Table 1 Phytochemical survey of *Calluna vulgaris* (L.) HULL.

No.	Compound Class	Compound	References
	Chromones		
1		5,7- Dihydroxychromone	(Simon et al., 1994)
2		5,7- Dihydroxychromone-7-ß-D-glucoside	(Simon et al., 1994)
	Flavonoids		
	Flavones		
	Flavone glycosides		
3		Apigenin-7-(2´´-acetyl-6´´-methylglucuronid) [f]	(Allais et al., 1991)
4		Apigenin hexuronolactone [a, h]	(Monschein et al., 2008)
	Flavonols		
5		Herbacetin	(Jalal et al., 1982; Olechnowicz-Stepien et al., 1978; Shelyuto et al., 1977)
6		Kaempferol [s]	(Harborne et al., 1973; Jalal et al., 1982; Mantilla et al., 1975; Olechnowicz-Stepien et al., 1978)
7		Myricetin	(Hoppe, 1975; Jalal et al., 1982; Olechnowicz-Stepien et al., 1978)
8		Quercetin [f, s]	(Jalal et al., 1982; Mantilla et al., 1975; Olechnowicz-Stepien et al., 1978; Wehmer, 1931)
9		Isoscutellarein	(Shelyuto et al., 1975)

Table 1 continued

	Flavonol glycosides		
10		Isorhamnetin-3-*O*-galactoside [h]	(Ersoz et al., 1997)
11		Kaempferol-3-*O*-arabinoside [s]	(Jalal et al., 1982; Simon et al., 1993a)
12		Kaempferol-3-O-diacetylarabinohexoside [a, h]	(Monschein et al., 2008)
13		Kaempferol-3-*O*-(6´´-p-coumaroyl)-β-D-glucoside (Tiliroside) [a]	(Monschein et al., 2008)
14		Kaempferol-3-*O*-galactoside [s]	(Jalal et al., 1982; Orhan et al., 2007)
15		Kaempferol-3-*O*-glucoside [s]	(Jalal et al., 1982)
16		Kaempferol-3-[2´´´,3´´´,5´´´-triacetyl-α-L-arabinofuranosyl-(1→6)-ß-D-glucoside] [f]	(Allais et al., 1991; Simon et al., 1993a)
17		Kaempferol-3-[2´´´,3´´´,4´´´-triacetyl-α-L-arabinopyranosyl-(1→6)-ß-D-glucoside] [f]	(Allais et al., 1991; Simon et al., 1993b)
18		3-Methoxy-5,7-dihydroxyflavone-7-O-glucoside [f] (Galangin-3-methylether-7-glucoside; 3-Methylgalangin)	(Olechnowicz-Stepien et al., 1978)
19		3,5,7,8,4´- Pentahydroxyflavon-8-O-gentiobioside [f, r, s] (Herbacetin-8-O-gentiobiosid)	(Olechnowicz-Stepien et al., 1978)
20		3,5,7,8,4´-Pentahydroxyflavon-4´-O-*ß*-D-glucoside [h] (Herbacetin-4´-O-glucoside)	(Ersoz et al., 1997)
21		3,5,7,8,4´- Pentahydroxyflavon-8-O-glucoside [f, r, s] (Herbacetin-8-O-glucoside)	(Olechnowicz-Stepien et al., 1978)
22		Quercetin-3-*O*-arabinoside *(f)* (Avicularin)	(Olechnowicz-Stepien et al., 1978)
23		Quercetin-3-*O*-arabinoside *(p)* [s] (Guajaverin)	(Jalal et al., 1982)
24		Quercetin-3-*O*-diacetylarabinohexoside [a, h]	(Monschein et al., 2008)

Table 1 continued

25		Quercetin-3-*O*-galactoside [f, h, r, s] (Hyperosid; Hyperin)	(Jalal et al., 1982; Olechnowicz-Stepien et al., 1978)
26		Qercetin-3-*O*-glucoside [f, r, s] (Isoquercitrin)	(Jalal et al., 1982)
27		Quercetin-3-*O*-monoacetylarabinohexoside [a, h]	(Monschein et al., 2008)
28		Quercetin-3-*O*-tetraacetylarabinohexoside [a, h]	(Monschein et al., 2008)
29		Quercetin-3-[2´´´,3´´´,5´´´-triacetyl-α-L-arabinofuranosyl-(1→6)-ß-D-glucoside]	(Simon et al., 1993b)
30		Quercetin-3-[2´´´,3´´´,4´´´-triacetyl-α-L-arabinopyranosyl-(1→6)-ß-D-galactoside]	(Simon et al., 1994)
31		Quercetin-3-[2´´´,3´´´,4´´´-triacetyl-α-L-arabinopyranosyl-(1→6)-ß-D-glucoside]	(Simon et al., 1993a)
	Dihydro-flavonols		
32		Dihydroherbacetin	(Jalal et al., 1982; Olechnowicz-Stepien et al., 1978; Shelyuto et al., 1977)
	Dihydro-flavonol glycosides		
33		3,5,7,8,4´- Pentahydroxyflavanone-8-(2´´-acetylglucosid) [f] (2´´-Acetylcallunin)	(Allais et al., 1995)
34		3,5,7,3´,4´-Pentahydroxyflavanone-3-*O*-glucoside [f] (Taxifolin-3-*O*-glucoside)	(Olechnowicz-Stepien et al., 1978)
35		3,5,7,8,4´- Pentahydroxyflavanone-8-glucoside [f, h , r, s] (Callunin)	(Jalal et al., 1982; Lamer-Zarawska et al., 1986; Simon et al., 1993a)
36		5,7,8,4´- Tetrahydroxyflavanon-8-glucosid [f] (3-Desoxycallunin)	(Allais et al., 1995)

Table 1 continued

	Catechins, Procyanidins		
37		(+)-Catechin [s, r]	(Jalal et al., 1982)
38		(-)-Epicatechin [s, r]	(Jalal et al., 1982)
39		Procyanidine A_2 [r]	(Jalal et al., 1982)
40		Procyanidine B_1 [s, r]	(Jalal et al., 1982)
41		Procyanidine B_2 [s, r]	(Jalal et al., 1982)
42		Procyanidine B_3 [s, r]	(Jalal et al., 1982)
43		Procyanidine B_4 [s]	(Jalal et al., 1982)
44		Procyanidine B_5 [s]	(Jalal et al., 1982)
45		Procyanidine C_1 [s]	(Jalal et al., 1982)
46		Procyanidine D_1 [s, r]	(Jalal et al., 1982; Shelyuto et al., 1975)
	Anthocyanidins		
47		Cyanidin-3-*O*-glucoside	(Allais et al., 1995)
	Phenolic acids		
	Hydroxy-cinnamic acids		
48		Caffeic acid [s]	(Jalal et al., 1982)
49		5-*O*-Caffeoylquinic acid [h, s, r] (Chlorogenic acid)	(Jalal et al., 1982; Mantilla et al., 1975)
50		3-O-Caffeoylquinic acid [h]	(Monschein et al., 2008)
51		p-Coumaric acid	(Mantilla et al., 1975)
52		p-Coumaroylquinic acid [a, h]	(Monschein et al., 2008)
53		Ferulic acid [r]	(Jalal et al., 1982)
54		Isochlorogenic acid [s, b]	(Jalal et al., 1982)

Table 1 continued

	Hydroxybenzoic acids		
55		5-Hydroxysalicylic acid (Gentisic acid)	(Mantilla et al., 1975)
56		Protocatechuic acid	(Mantilla et al., 1975)
57		Syringic acid	(Mantilla et al., 1975)
58		Vanillic acid [r]	(Mantilla et al., 1975)
	Phenols and phenol glycosides		
59		3,5- Dihydroxytoluene [s] (Orcin; Orcinol)	(Ballester et al., 1972; Jalal et al., 1982)
60		Hydroquinone	(Mantilla et al., 1975; Sticher et al., 1979)
61		Hydroquinone-*ß*-D-glucopyranoside [s, r] (Arbutin)	(Jalal et al., 1982; Sticher et al., 1979)
62		1-[4-(β-D-glucopyranosyloxy)-2-hydroxy-6-methoxyphenyl]-ethanone [a, h] (Rodiolinozide, Annphenone)	(Monschein et al., 2008)
63		Orcinol-*ß*-D-glucoside [s, r] (Sakakin)	(Ballester et al., 1972; Jalal et al., 1982; Mantilla et al., 1975; Sticher et al., 1979)
	Lipids		
	Fatty acids		
64		Myristic acid (n-Tetradecoic acid)	(Olechnowicz-Stepien et al., 1982)
65		n-Nonacosane [f]	(Olechnowicz-Stepien et al., 1982)
66		Octadecenoic acid	(Olechnowicz-Stepien et al., 1982)

Table 1 continued

67		Octadecatrienoic acid	(Olechnowicz-Stepien et al., 1982)
68		Octadecadienoic acid	(Olechnowicz-Stepien et al., 1982)
69		Palmitic acid	(Olechnowicz-Stepien et al., 1982)
70		Stearic acid	(Olechnowicz-Stepien et al., 1982)
	Steroids and triterpenes		
71		α-Amyrine	(Olechnowicz-Stepien et al., 1982)
72		ß-Sitosterol [f]	(Olechnowicz-Stepien et al., 1982)
73		Ursolic acid [f]	(Olechnowicz-Stepien et al., 1982)
	Other compounds		
74		Ascorbic acid	(Jones et al., 1983)
75		Mucilages	(Tamas et al., 2005)

[a] Identified in this species for the first time; [b] isomer not defined (identical with **50** ?).

If available, information on the plant part was included: [f] flowers; [h] herbal parts; [s] shoots/stems; [r] roots. LC-ESI-MS analysis (Monschein et al. (2008)): column Synergi Hydro-RP (150 x 2 mm), gradient: A = 0.5 % acetic acid, B = 0.5 % acetic acid in acetonitril-water (1:1), 10% B (0-11 min), 10-25% B (13-60 min), 25-100% B (60-92 min), 0.3 ml/min; ESI-MS, negative mode, 4.5 kV source voltage, 330 °C capillary temperature

Table 2 ^{1}H and ^{13}C NMR data (400 and 100 MHz, resp.) of rodiolinozide (**62**) (pyridine-d_5, 23 °C)

Pos.	δ_C	δ_H	HMBC correlations
1	33.1	2.53 (s)	$H_1 \rightarrow C_2$
2	203.5	-	
1`	107.0	-	
2`	167.4	-	
3`	97.4	6.73 (d, *J* = 2.4 Hz)	$H_{3`} \rightarrow C_{2`}, C_{3`}, C_{4`}$
4`	164.9	-	
5`	92.2	6.41 (d, *J* = 2.4 Hz)	$H_{5`} \rightarrow C_{4`}, C_{6`}, C_{1`}$
6`	163.3	-	
7`	55.9	3.70 (s)	$H_{7`} \rightarrow C_{6`}$
1``	101.8	5.76 d, *J* = 7.2 Hz	$H_{1``} \rightarrow C_{4`}$
2``	75.0	4.36 (m)	
3``	78.9	4.36 (m)	
4``	71.3	4.33 (m)	
5``	79.6	4.15 (m)	
6``	62.6	4.55 (br d, *J* = 11 Hz)	
		4.37 (m)	

Table 3: Content of major phenolic compounds in herbs of *Calluna vulgaris* of samples collected at different altitudes during four growing periods[a]

Compound	**Samples**	**800 m**	**1000 m**	**1500 m**
Chlorogenic acid (**49**)	year 1[c] (n = 11)	0.95 ± 0.22 a	1.00 ± 0.15 a	0.92 ± 0.25 a
	year 2[c] (n = 11-13)	0.94 ± 0.20 a	1.05 ± 0.14 a	1.11 ± 0.16 a
	year 3[d] (n = 12-13)	1.20 ± 0.16 a[**]	0.97 ± 0.19 b[**]	1.18 ± 0.21 c
	year 4[d] (n = 10-12)	0.99 ± 0.23 a	0.83 ± 0.16 a	0.97 ± 0.26 a
	Mean	**1.02 ± 0.10**	**0.96 ± 0.08**	**1.04 ± 0.10**
Callunin[e] (**35**)	year 1 (n = 11)	1.23 ± 0.14 a	1.23 ± 0.19 a	1.10 ± 0.27 a
	year 2 (n = 11-13)	1.23 ± 0.24 a	1.18 ± 0.30 a	1.24 ± 0.24 a
	year 3 (n = 12-13)	0.91 ± 0.35 a	1.04 ± 0.24 a	1.19 ± 0.28 a
	year 4[b] (n = 10-12)	0.87 ± 0.32 a[*]	1.04 ± 0.29 a,b	1.16 ± 0.23 b
	Mean	**1.06 ± 0.17**	**1.12 ± 0.08**	**1.17 ± 0.05**
Hyperoside (**25**)	year 1[b] (n = 11)	0.38 ± 0.06 a[***,***]	0.63 ± 0.14 b	1.31 ± 0.43 c
	year 2[b] (n = 11-13)	0.44 ± 0.11 a[*,**]	0.57 ± 0.15 b[***]	1.33 ± 0.32 c
	year 3[b] (n = 12-13)	0.50 ± 0.12 a[***]	0.55 ± 0.12 a[***]	1.23 ± 0.29 b
	year 4[b] (n = 10-12)	0.42 ± 0.19 a[***]	0.43 ± 0.09 a[***]	0.96 ± 0.28 b
	Mean	**0.43 ± 0.04**	**0.54 ± 0.07**	**1.21 ± 0.15**
Isoquercitrin (**26**)	year 1[b] (n = 11)	0.09 ± 0.01 a[***,***]	0.15 ± 0.03 b	0.28 ± 0.11 c
	year 2[b] (n = 11-13)	0.13 ± 0.03 a[***]	0.14 ± 0.03 a[***]	0.29 ± 0.09 b
	year 3[b] (n = 12-13)	0.12 ± 0.04 a[***]	0.13 ± 0.05 a[***]	0.31 ± 0.11 b
	year 4[b] (n = 10-12)	0.08 ± 0.05 a[***]	0.08 ± 0.03 a[***]	0.20 ± 0.09 b
	Mean	**0.10 ± 0.02**	**0.12 ± 0.03**	**0.27 ± 0.04**

Table 3 continued

Kaempferol-3-*O*-galactoside (**14**)	year 1 (n = 11)	0.25 ± 0.04 a***,***	0.35 ± 0.08 b***	0.43 ± 0.10 c
	year 2 (n = 11-13)	0.28 ± 0.07 a***,***	0.33 ± 0.04 b***	0.45 ± 0.07 c
	year 3[b] (n = 12-13)	0.19 ± 0.04 a**	0.21 ± 0.09 a**	0.31 ± 0.10 b
	year 4[b] (n = 10-12)	0.16 ± 0.02 a***	0.16 ± 0.07 a***	0.26 ± 0.11 b
	Mean	**0.22 ± 0.05**	**0.26 ± 0.08**	**0.36 ± 0.08**

[a] Statistically significant differences are marked with different letters. Level of significance is indicated by asterisks * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Results at 800 m: asterisks are correlated to the comparison of the values from 800/1000 m before the comma and to the values from 800/1500 m after the comma. Results at 1000 m: asterisks are correlated to the comparison of the values from 1000/1500 m.

n = number of samples/altitude/year.

[b] Kruskal-Wallis test, broken down by vegetation period. In all other cases samples of one altitude were pooled and analyzed by two-way analysis in a 2 × 2 design (year 1, 2) or equal variance test (year 3, 4).

[c] Data from Rieger et al. (2008).

[d] Data from (Monschein, 2009; Monschein et al., 2009).

[e] In Rieger et al. (2008) erroneously designated as taxifolin-3-O-glucoside

Abstract

Calluna vulgaris (L.) Hull. (heather) is the only species within the monotypic genus *Calluna* (Ericaceae). It is a dominant species of heather communities and can be found in most parts of Europe and Northern America from lowland up to alpine regions. Common heather is traditionally used to treat urinary tract disturbances and inflammatory related disorders.

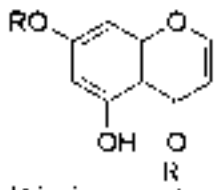
This review covers the current knowledge on phytochemical investigations of *C. vulgaris* which revealed a complex pattern of flavonoid glycosides including acetylated compounds as well as other classes of phenolics (chromones, procyanidins, simple phenols, etc.) Recently, an acetophenone (rodiolinozid) could be identified.

Its occurance over a wide range of altitudinal zones makes *C. vulgaris* an interesting species to study the variation of its metabolic profiles in wild populations growing under different climatic conditions. Within phenolic compounds flavonols showed significant differences in samples collected at different altitudes with increased levels of quercetin glycosides at higher altitudes whereas no significant correlation could be found for caffeoyl quinic acids and the dihydroflavonol glycoside callunin.

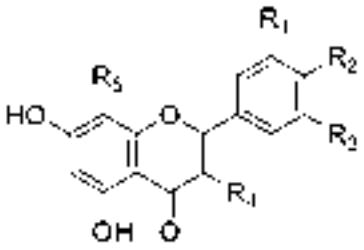
Broadening the scope of such investigations by looking at different species and different geographical areas should give a more concrete picture which phenolic compounds, if any, are suitable markers to observe adaptive processes in high altitude plants. Furthermore, investigations on the specific patterns of phenolics at cellular and subcellular level and their variation due to factors like enhanced solar radiation and low temperature should be expanded.

Figure1

Click here to download high resolution image

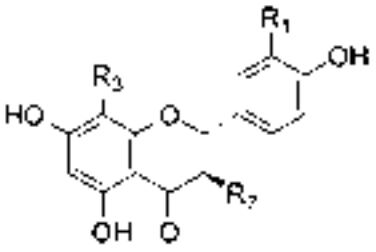


	R
5,7-Dihydroxychromone (1)	H
5,7-Dihydroxychromone-7-*β*-D-glucoside (2)	*β*-D-glucoside



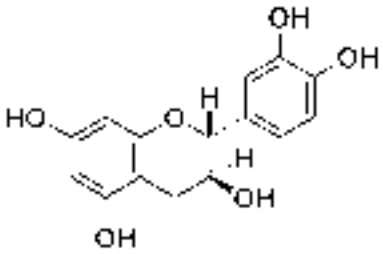
Flavones, flavonols

	R_1	R_2	R_3	R_4	R_5
Apigenin	H	OH	H	H	H
Herbacetin (5)	H	OH	H	OH	OH
Isorhamnetin	OCH_3	OH	H	OH	H
Kaempferol (6)	H	OH	H	OH	H
Myricetin (7)	OH	OH	OH	OH	H
Quercetin (8)	OH	OH	H	OH	H

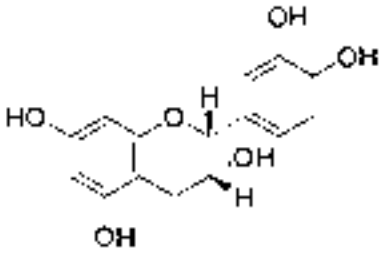


Dihydroflavonols

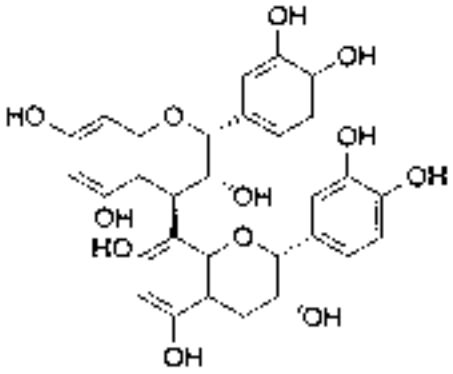
	R_1	R_2	R_3
Taxifolin-3-*O*-glucoside (34)	glucoside	OH	H
Taxifolin (35)	H	OH	glucoside
3-Deoxy-taxifolin (36)	H	H	glucoside



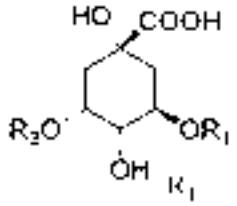
(+)-Catechin (37)



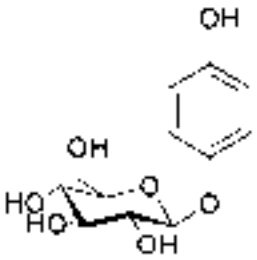
(−)-Epicatechin (38)



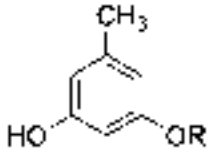
Proanthocyanidin B$_3$ (41)



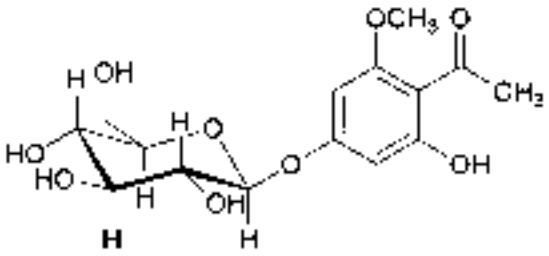
	R_1	R_2
3-*O*-Caffeoyl-quinic acid (50)	caffeoyl	H
5-*O*-Caffeoyl-quinic acid (49)	H	caffeoyl



Arbutin (61)



	R
Orcinol (59)	H
Orcinol-[4-D-glucoside (63)	*β*-D-glucoside



Rodiolarioside (62)

Fugure2

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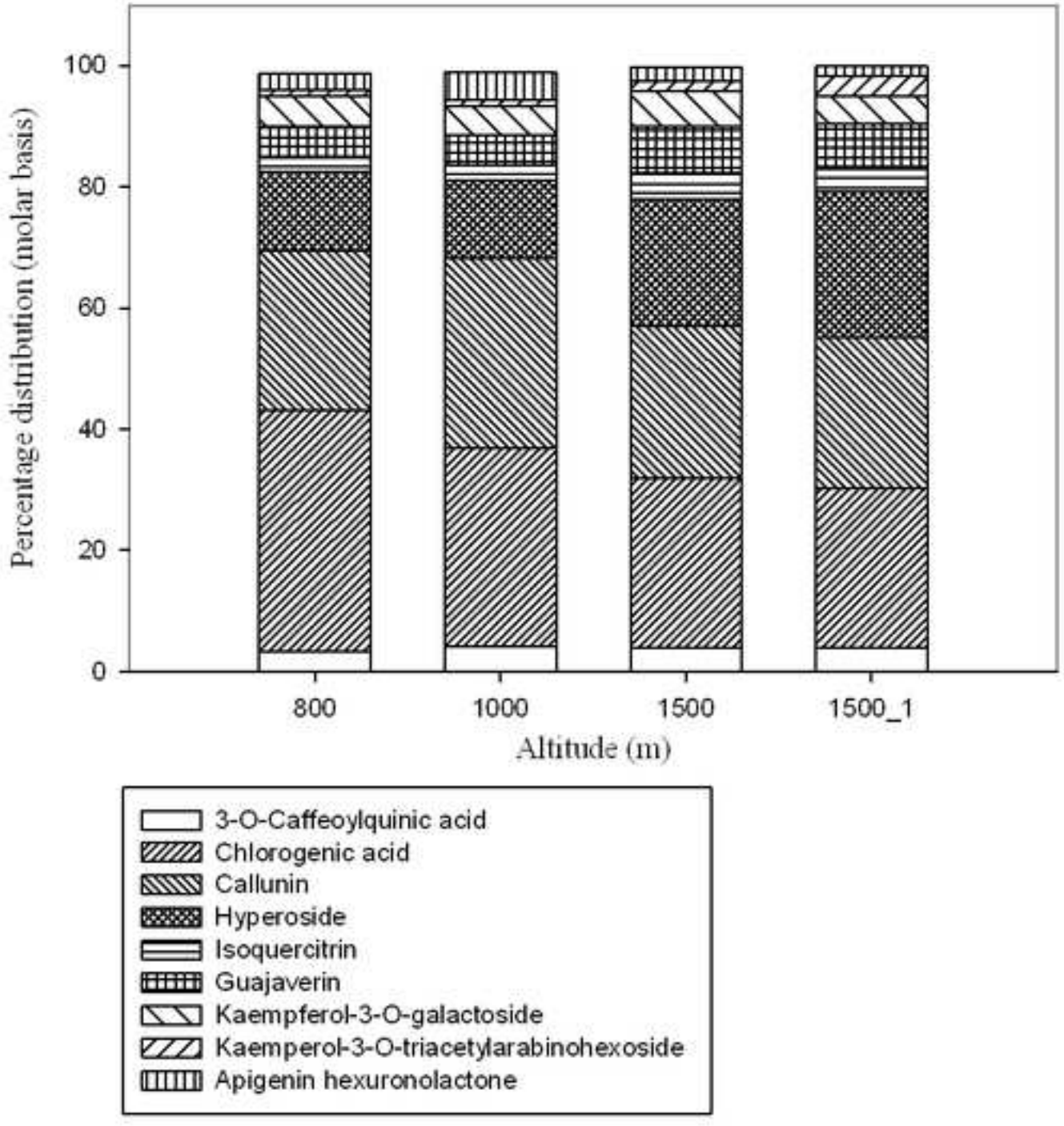

Table 1 Phytochemical survey of *Calluna vulgaris* (L.) HULL.

No.	Compound Class	Compound	References
	Chromones		
1		5,7- Dihydroxychromone	(Simon et al., 1994)
2		5,7- Dihydroxychromone-7-ß-D-glucoside	(Simon et al., 1994)
	Flavonoids		
	Flavones		
	Flavone glycosides		
3		Apigenin-7-(2´´-acetyl-6´´-methylglucuronid) [f]	(Allais et al., 1991)
4		Apigenin hexuronolactone [a, h]	(Monschein et al., 2008)
	Flavonols		
5		Herbacetin	(Jalal et al., 1982; Olechnowicz-Stepien et al., 1978; Shelyuto et al., 1977)
6		Kaempferol [s]	(Harborne et al., 1973; Jalal et al., 1982; Mantilla et al., 1975; Olechnowicz-Stepien et al., 1978)
7		Myricetin	(Hoppe, 1975; Jalal et al., 1982; Olechnowicz-Stepien et al., 1978)
8		Quercetin [f, s]	(Jalal et al., 1982; Mantilla et al., 1975; Olechnowicz-Stepien et al., 1978; Wehmer, 1931)
9		Isoscutellarein	(Shelyuto et al., 1975)

Table 1 continued

	Flavonol glycosides		
10		Isorhamnetin-3-*O*-galactoside [h]	(Ersoz et al., 1997)
11		Kaempferol-3-*O*-arabinoside [s]	(Jalal et al., 1982; Simon et al., 1993a)
12		Kaempferol-3-O-diacetylarabinohexoside [a, h]	(Monschein et al., 2008)
13		Kaempferol-3-*O*-(6´´-p-coumaroyl)-β-D-glucoside (Tiliroside) [a]	(Monschein et al., 2008)
14		Kaempferol-3-*O*-galactoside [s]	(Jalal et al., 1982; Orhan et al., 2007)
15		Kaempferol-3-*O*-glucoside [s]	(Jalal et al., 1982)
16		Kaempferol-3-[2´´´,3´´´,5´´´-triacetyl-α-L-arabinofuranosyl-(1→6)-ß-D-glucoside] [f]	(Allais et al., 1991; Simon et al., 1993a)
17		Kaempferol-3-[2´´´,3´´´,4´´´-triacetyl-α-L-arabinopyranosyl-(1→6)-ß-D-glucoside] [f]	(Allais et al., 1991; Simon et al., 1993b)
18		3-Methoxy-5,7-dihydroxyflavone-7-O-glucoside [f] (Galangin-3-methylether-7-glucoside; 3-Methylgalangin)	(Olechnowicz-Stepien et al., 1978)
19		3,5,7,8,4´- Pentahydroxyflavon-8-O-gentiobioside [f, r, s] (Herbacetin-8-O-gentiobiosid)	(Olechnowicz-Stepien et al., 1978)
20		3,5,7,8,4´-Pentahydroxyflavon-4´-O-*ß*-D-glucoside [h] (Herbacetin-4´-O-glucoside)	(Ersoz et al., 1997)
21		3,5,7,8,4´- Pentahydroxyflavon-8-O-glucoside [f, r, s] (Herbacetin-8-O-glucoside)	(Olechnowicz-Stepien et al., 1978)
22		Quercetin-3-*O*-arabinoside *(f)* (Avicularin)	(Olechnowicz-Stepien et al., 1978)
23		Quercetin-3-*O*-arabinoside *(p)* [s] (Guajaverin)	(Jalal et al., 1982)
24		Quercetin-3-*O*-diacetylarabinohexoside [a, h]	(Monschein et al., 2008)

Table 1 continued

25		Quercetin-3-*O*-galactoside [f, h, r, s] (Hyperosid; Hyperin)	(Jalal et al., 1982; Olechnowicz-Stepien et al., 1978)
26		Qercetin-3-*O*-glucoside [f, r, s] (Isoquercitrin)	(Jalal et al., 1982)
27		Quercetin-3-*O*-monoacetylarabinohexoside [a, h]	(Monschein et al., 2008)
28		Quercetin-3-*O*-tetraacetylarabinohexoside [a, h]	(Monschein et al., 2008)
29		Quercetin-3-[2´´´,3´´´,5´´´-triacetyl-α-L-arabinofuranosyl-(1→6)-ß-D-glucoside]	(Simon et al., 1993b)
30		Quercetin-3-[2´´´,3´´´,4´´´-triacetyl-α-L-arabinopyranosyl-(1→6)-ß-D-galactoside]	(Simon et al., 1994)
31		Quercetin-3-[2´´´,3´´´,4´´´-triacetyl-α-L-arabinopyranosyl-(1→6)-ß-D-glucoside]	(Simon et al., 1993a)
	Dihydro-flavonols		
32		Dihydroherbacetin	(Jalal et al., 1982; Olechnowicz-Stepien et al., 1978; Shelyuto et al., 1977)
	Dihydro-flavonol glycosides		
33		3,5,7,8,4´- Pentahydroxyflavanone-8-(2´´-acetylglucosid) [f] (2´´-Acetylcallunin)	(Allais et al., 1995)
34		3,5,7,3´,4´-Pentahydroxyflavanone-3-*O*-glucoside [f] (Taxifolin-3-*O*-glucoside)	(Olechnowicz-Stepien et al., 1978)
35		3,5,7,8,4´- Pentahydroxyflavanone-8-glucoside [f, h , r, s] (Callunin)	(Jalal et al., 1982; Lamer-Zarawska et al., 1986; Simon et al., 1993a)
36		5,7,8,4´- Tetrahydroxyflavanon-8-glucosid [f] (3-Desoxycallunin)	(Allais et al., 1995)

Table 1 continued

	Catechins, Procyanidins		
37		(+)-Catechin [s, r]	(Jalal et al., 1982)
38		(-)-Epicatechin [s, r]	(Jalal et al., 1982)
39		Procyanidine A_2 [r]	(Jalal et al., 1982)
40		Procyanidine B_1 [s, r]	(Jalal et al., 1982)
41		Procyanidine B_2 [s, r]	(Jalal et al., 1982)
42		Procyanidine B_3 [s, r]	(Jalal et al., 1982)
43		Procyanidine B_4 [s]	(Jalal et al., 1982)
44		Procyanidine B_5 [s]	(Jalal et al., 1982)
45		Procyanidine C_1 [s]	(Jalal et al., 1982)
46		Procyanidine D_1 [s, r]	(Jalal et al., 1982; Shelyuto et al., 1975)
	Anthocyanidins		
47		Cyanidin-3-*O*-glucoside	(Allais et al., 1995)
	Phenolic acids		
	Hydroxy-cinnamic acids		
48		Caffeic acid [s]	(Jalal et al., 1982)
49		5-*O*-Caffeoylquinic acid [h, s, r] (Chlorogenic acid)	(Jalal et al., 1982; Mantilla et al., 1975)
50		3-O-Caffeoylquinic acid [h]	(Monschein et al., 2008)
51		p-Coumaric acid	(Mantilla et al., 1975)
52		p-Coumaroylquinic acid [a, h]	(Monschein et al., 2008)
53		Ferulic acid [r]	(Jalal et al., 1982)
54		Isochlorogenic acid [s, b]	(Jalal et al., 1982)

Table 1 continued

	Hydroxybenzoic acids		
55		5-Hydroxysalicylic acid (Gentisic acid)	(Mantilla et al., 1975)
56		Protocatechuic acid	(Mantilla et al., 1975)
57		Syringic acid	(Mantilla et al., 1975)
58		Vanillic acid [r]	(Mantilla et al., 1975)
	Phenols and phenol glycosides		
59		3,5- Dihydroxytoluene [s] (Orcin; Orcinol)	(Ballester et al., 1972; Jalal et al., 1982)
60		Hydroquinone	(Mantilla et al., 1975; Sticher et al., 1979)
61		Hydroquinone-*ß*-D-glucopyranoside [s, r] (Arbutin)	(Jalal et al., 1982; Sticher et al., 1979)
62		1-[4-(β-D-glucopyranosyloxy)-2-hydroxy-6-methoxyphenyl]-ethanone [a, h] (Rodiolinozide, Annphenone)	(Monschein et al., 2008)
63		Orcinol-*ß*-D-glucoside [s, r] (Sakakin)	(Ballester et al., 1972; Jalal et al., 1982; Mantilla et al., 1975; Sticher et al., 1979)
	Lipids		
	Fatty acids		
64		Myristic acid (n-Tetradecoic acid)	(Olechnowicz-Stepien et al., 1982)
65		n-Nonacosane [f]	(Olechnowicz-Stepien et al., 1982)
66		Octadecenoic acid	(Olechnowicz-Stepien et al., 1982)

Table 1 continued

67		Octadecatrienoic acid	(Olechnowicz-Stepien et al., 1982)
68		Octadecadienoic acid	(Olechnowicz-Stepien et al., 1982)
69		Palmitic acid	(Olechnowicz-Stepien et al., 1982)
70		Stearic acid	(Olechnowicz-Stepien et al., 1982)
	Steroids and triterpenes		
71		α-Amyrine	(Olechnowicz-Stepien et al., 1982)
72		ß-Sitosterol [f]	(Olechnowicz-Stepien et al., 1982)
73		Ursolic acid [f]	(Olechnowicz-Stepien et al., 1982)
	Other compounds		
74		Ascorbic acid	(Jones et al., 1983)
75		Mucilages	(Tamas et al., 2005)

[a] Identified in this species for the first time; [b] isomer not defined (identical with **50** ?).

If available, information on the plant part was included: [f] flowers; [h] herbal parts; [s] shoots/stems; [r] roots. LC-ESI-MS analysis (Monschein et al. (2008)): column Synergi Hydro-RP (150 x 2 mm), gradient: A = 0.5 % acetic acid, B = 0.5 % acetic acid in acetonitril-water (1:1), 10% B (0-11 min), 10-25% B (13-60 min), 25-100% B (60-92 min), 0.3 ml/min; ESI-MS, negative mode, 4.5 kV source voltage, 330 °C capillary temperature

Table 2 ^{1}H and ^{13}C NMR data (400 and 100 MHz, resp.) of rodiolinozide (**62**) (pyridine-d_5, 23 °C)

Pos.	δ_C	δ_H	HMBC correlations
1	33.1	2.53 (s)	$H_1 \rightarrow C_2$
2	203.5	-	
1`	107.0	-	
2`	167.4	-	
3`	97.4	6.73 (d, *J* = 2.4 Hz)	$H_{3`} \rightarrow C_{2`}, C_{3`}, C_{4`}$
4`	164.9	-	
5`	92.2	6.41 (d, *J* = 2.4 Hz)	$H_{5`} \rightarrow C_{4`}, C_{6`}, C_{1`}$
6`	163.3	-	
7`	55.9	3.70 (s)	$H_{7`} \rightarrow C_{6`}$
1``	101.8	5.76 d, *J* = 7.2 Hz	$H_{1``} \rightarrow C_{4`}$
2``	75.0	4.36 (m)	
3``	78.9	4.36 (m)	
4``	71.3	4.33 (m)	
5``	79.6	4.15 (m)	
6``	62.6	4.55 (br d, *J* = 11 Hz)	
		4.37 (m)	

Table 3: Content of major phenolic compounds in herbs of *Calluna vulgaris* of samples collected at different altitudes during four growing periods[a]

Compound	**Samples**	**800 m**	**1000 m**	**1500 m**
Chlorogenic acid (**49**)	year 1[c] (n = 11)	0.95 ± 0.22 a	1.00 ± 0.15 a	0.92 ± 0.25 a
	year 2[c] (n = 11-13)	0.94 ± 0.20 a	1.05 ± 0.14 a	1.11 ± 0.16 a
	year 3[d] (n = 12-13)	1.20 ± 0.16 a[**]	0.97 ± 0.19 b[**]	1.18 ± 0.21 c
	year 4[d] (n = 10-12)	0.99 ± 0.23 a	0.83 ± 0.16 a	0.97 ± 0.26 a
	Mean	**1.02 ± 0.10**	**0.96 ± 0.08**	**1.04 ± 0.10**
Callunin[e] (**35**)	year 1 (n = 11)	1.23 ± 0.14 a	1.23 ± 0.19 a	1.10 ± 0.27 a
	year 2 (n = 11-13)	1.23 ± 0.24 a	1.18 ± 0.30 a	1.24 ± 0.24 a
	year 3 (n = 12-13)	0.91 ± 0.35 a	1.04 ± 0.24 a	1.19 ± 0.28 a
	year 4[b] (n = 10-12)	0.87 ± 0.32 a[*]	1.04 ± 0.29 a,b	1.16 ± 0.23 b
	Mean	**1.06 ± 0.17**	**1.12 ± 0.08**	**1.17 ± 0.05**
Hyperoside (**25**)	year 1[b] (n = 11)	0.38 ± 0.06 a[***,***]	0.63 ± 0.14 b	1.31 ± 0.43 c
	year 2[b] (n = 11-13)	0.44 ± 0.11 a[*,**]	0.57 ± 0.15 b[***]	1.33 ± 0.32 c
	year 3[b] (n = 12-13)	0.50 ± 0.12 a[***]	0.55 ± 0.12 a[***]	1.23 ± 0.29 b
	year 4[b] (n = 10-12)	0.42 ± 0.19 a[***]	0.43 ± 0.09 a[***]	0.96 ± 0.28 b
	Mean	**0.43 ± 0.04**	**0.54 ± 0.07**	**1.21 ± 0.15**
Isoquercitrin (**26**)	year 1[b] (n = 11)	0.09 ± 0.01 a[***,***]	0.15 ± 0.03 b	0.28 ± 0.11 c
	year 2[b] (n = 11-13)	0.13 ± 0.03 a[***]	0.14 ± 0.03 a[***]	0.29 ± 0.09 b
	year 3[b] (n = 12-13)	0.12 ± 0.04 a[***]	0.13 ± 0.05 a[***]	0.31 ± 0.11 b
	year 4[b] (n = 10-12)	0.08 ± 0.05 a[***]	0.08 ± 0.03 a[***]	0.20 ± 0.09 b
	Mean	**0.10 ± 0.02**	**0.12 ± 0.03**	**0.27 ± 0.04**

Table 3 continued

Kaempferol-3-*O*-galactoside (**14**)	year 1 (n = 11)	0.25 ± 0.04 a***,***	0.35 ± 0.08 b***	0.43 ± 0.10 c
	year 2 (n = 11-13)	0.28 ± 0.07 a***,***	0.33 ± 0.04 b***	0.45 ± 0.07 c
	year 3[b] (n = 12-13)	0.19 ± 0.04 a**	0.21 ± 0.09 a**	0.31 ± 0.10 b
	year 4[b] (n = 10-12)	0.16 ± 0.02 a***	0.16 ± 0.07 a***	0.26 ± 0.11 b
	Mean	**0.22 ± 0.05**	**0.26 ± 0.08**	**0.36 ± 0.08**

[a] Statistically significant differences are marked with different letters. Level of significance is indicated by asterisks * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Results at 800 m: asterisks are correlated to the comparison of the values from 800/1000 m before the comma and to the values from 800/1500 m after the comma. Results at 1000 m: asterisks are correlated to the comparison of the values from 1000/1500 m.

n = number of samples/altitude/year.

[b] Kruskal-Wallis test, broken down by vegetation period. In all other cases samples of one altitude were pooled and analyzed by two-way analysis in a 2 × 2 design (year 1, 2) or equal variance test (year 3, 4).

[c] Data from Rieger et al. (2008).

[d] Data from (Monschein, 2009; Monschein et al., 2009).

[e] In Rieger et al. (2008) erroneously designated as taxifolin-3-O-glucoside