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Phytochemical characterization and functional investigation of *Rhododendron ferrugineum* L.

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Rhododendron ferrugineum L. is a subalpine shrub found throughout the Pyrenees and the European Alps at elevations of 1700 to 2300 m. The leaves were traditionally used as aqueous decocts for several indications, especially rheumatism, blood pressure, muscle and metabolic diseases. From the phytochemical point of view the description of the plant is incomplete and rudimental. The fact that a potential toxicity due to hydroquinone and andromedotoxin and its derivatives could not be excluded, resulted in the issuance of a negative monograph and the complete ban of any therapeutic use of *R. ferrugineum* L.

Therefore the aim of this work was a broad phytochemical investigation of the polar and semipolar compounds of the leaves of *R. ferrugineum* L., including functional tests of different extracts on keratinocytes and the establishment of a UPLC-method for quality control of water-methanol-extracts from *R. ferrugineum* L.

The main focus of the phytochemical investigation was on the volatile oil, flavonoids and tannins and carbohydrates.

The volatile oil was obtained via steam distillation according to Ph. Eur. and investigated by GC-MS. The content was determined as 7.4 mL/kg. 16 main compounds could be identified all belonging to the class of mono- and sesquiterpens. α-pinene, *ar*-/γ-curcumene, γ-muurolene and α-/β-selinene were found to be quantitatively dominant.

The isolation of flavonoids and tannins was performed by different chromatographic methods including column chromatography, liquid chromatography and thin layer chromatography using different stationary phases. Structure elucidation was done by means of 1D- and 2D-NMR-spectroscopy, CD-spectroscopy, mass spectrometry and polarimetry.

Concerning the flavonoids, ten flavonols, two flavonones and two dihydroflavonoids could be isolated and identified, including two new natural compounds and eleven flavonoids which have been reported for *R. ferrugineum* L. for the first time. 2R,3R-taxifolin 3-O-β-L-arabinopyranoside was identified as quantitatively dominant flavonoid in *R. ferrugineum* L.

The total tannin content of the dried leaves of *R. ferrugineum* L. was determined as 3.4 % calculated as pyrogallol (method Ph. Eur.). The proanthocyanidin pattern was investigated up to the trimeric compounds. It consisted mainly of A- and B-type procyanidins.

Within the framework of establishing and validating an UPLC-method for quality control of water-methanol-extracts from *R. ferrugineum* L., 2R,3R-taxifolin 3-O-β-L-arabinopyranoside, chlorogenic acid, hyperoside and isoquercitrin were identified as analytical marker compounds.

For characterization of carbohydrates from the leaves of *R. ferrugineum* L., an aqueous extract was analyzed by HPAEC-PAD. Glucose, fructose, sucrose, raffinose and stachyose were found to be dominant sugars. Additionally 3.0 % (m/m) of oligomeric and polymeric fructans were determined by an enzymatic assay. From an aqueous extract high-molecular polysaccharides (RPS) were isolated by ethanol precipitation (1.0 % (m/m)). The RPS were further fractionated and subfractions were characterized according to the respective molecular weight and protein and carbohydrate composition. One subfraction was exemplarily subjected to linkage determination of the different monomers by methylation analysis. Results obtained indicated the presence of type II arabinogalactan protein.

Functional testing via *in vitro* investigations on nonmalignant human epithelial keratinocytes showed for lipophilic extracts from *R. ferrugineum* L. a decrease of cell proliferation and vitality. An aqueous extract and and a mixture of procyanidin B1 to B4 (both in concentrations of 100 µg/mL) showed an increase of cell proliferation and vitality.

	Isolated compounds from the leaves of **R. ferrugineum** ***L.***
1	phloroacetophenone 4-O-β-D-glucopyranoside ◆
2	farrerol (6,8-dimethylnaringenin)
3	poriolin (poriol 7-O-β-D-glucopyranoside)
4	quercetin 3-O-(6′′-O-(2-methyl-1-oxobutyl))-β-D-glucopyranoside
5	chlorogenic acid
6	2R,3R taxifolin 3-O-(β)-L-arabinopyranoside ◆
7	quercetin 3-O-(6′′-O-acetyl)-β-D-glucopyranoside ◆
8	hyperoside (quercetin 3-O-β-D-galactopyranoside) ◆
9	isoquercitrin (quercetin 3-O-β-D-glucopyranoside)
10	guaijaverin (quercetin 3-O-α-L-arabinopyranoside)
11	2R,3R dihydromyricetin-3-O-(β)-L-arabinopyranoside
12	gossypetin 3-O-β-D-galactopyranoside
13	gossypetin 3-O-β-D-glucopyranoside
14	catechin
15	epicatechin
16	gallocatechin
17	epigallocatechin
18	myricetin 3′-O-β-D-xylopyranoside
19	quercetin
20	myricetin
21	procyanidin B1
22	procyanidin B2
23	procyanidin B3
24	procyanidin B4
25	procyanidin B5
26	procyanidin B6
27	procyanidin B7
28	procyanidin C1
29	epicatechin-(4β→8)-epicatechin-(4β→8)-catechin
30	cinnamtannin B1 (epicatechin-(2β→7, 4β→8)-epicatechin-(4β→8)-epicatechin)
31	pavetannin B6 (epicatechin-(2β→7, 4β→8)-epicatechin-(4β→8)-catechin)

new natural compounds, ◆ compounds which have already been discribed for *R. ferrugineum* L.