Flavonoids in glycerol macerates of *Ribes nigrum* L.

Kisseleff, N.[1], Schmid, T.[2], Ryffel, J.[2], Lapke, C.[2]

[1] University of Applied Science Zurich , Institute of Biotechnology, CH-8820 Wädenswil
[2] Spagyros AG, CH-3073 Gümligen

Introduction

In gemmotherapy, the buds of the black currant is used successfully. In the past, the macerates from *Ribes nigrum* (black currant) have attracted attention due to their health promoting benefits. Until now, only a few studies of the ingredients of the black currants have been made. The aim of this study was to identify the flavonoids of *Ribes nigrum*. Macerates from different batches had to be checked with HPTLC and HPLC. The essential ingredients had to be quantitatively determined. Other aims of this study were to gain more informations about the homogeneity of different batches, the stability of retain samples and the difference between the macerates of buds and leaves. The results should be compared with the literature.

Material and Methods

➢ **Test solutions**
Different batches of macerates from *Ribes nigrum* were used. All macerates were prepared according Ph.Eur. 7.0/2371 V.2.1.3. Following batches of the macerates of the buds were used, started at and sorted by the oldest date of production: 17020301, 17040303, 17060306, 17070401, 17080401, 17090504*, 17100501*, 17110305, 17120307, 17130701*. All batches except the marked ones were supplied by Spagyros, the others were supplied by Dixa. The batches 22.06.2012 and 29.07.2013 are macerates of the leaves.

➢ **Reference substances**
12 flavonoids from different suppliers.

➢ **Determination of flavonoids made with HPTLC**
Equipement:
HPTLC plates: silica gel 60 F254, 20*10 cm, Merck, lot No. HX389048; ADC 2, CAMAG; ATS 4, CAMAG; TLC Plate Heater III, CAMAG; TLC Visualizer, CAMAG; Desaga DS 20, Sysmex Digitana; visionCATS version 1.3.0.0, CAMAG.

Test solutions:
Mix 1 ml macerate with 2 ml tetrahydrofuran and 2 ml saturated sodium chloride solution, shake and use the upper phase for analysis. Filter.
Reference substances:
Dissolve 2.5-3.5 mg of each flavonoid in 10 ml methanol. Filter.
Application:
Test solution: 4 µl, reference solution: 2 µl as a band of 8 mm.
Mobile phase:
Ethyl acetate : acetic acid : formic acid : water (20 : 2 : 2 : 1 V/V/V/V), chamber saturation, developing distance: 70 mm.
Drying:
100°C for 3 minutes.
Derivatisation:
Spray the plate with 1 % diphenylboryloxy-ethylamine in methanol (m/m), dry by 100°C for 3 minutes, spray the plate with 5 % PEG 400 in methanol (V/V), dry in air by darkness for 30 minutes.
Detection:
Examine in ultraviolet light at 366 nm.

➢ **Determination of flavonoids made with HPLC**
Equipement:
Column: Nucleodur 100-3 C_{18} ec, N130330407, 125*4, MACHEREY-NAGEL; Photodiode Array Detector, Waters e2998; Empower 3 Waters-s4ddxw0, Waters
Test solutions:
Filter the macerates.
Reference substances:
Dissolve 2.5-3.5 mg of each flavonoid in 10 ml methanol. Filter.
Solvent:
A: phosphoric acid R:water R (3:1000 V/V);
B: phosphoric acid R:acetonitrile R (3:1000 V/V).
Gradient:

time (min)	mobile phase A (% V/V)
0.0-6.0	82
6.0-14.4	82→47
14.4-14.5	47→3
14.5-25.3	3→82
25.3-25.7	82

Flow: 0.6 ml/min
Injection volume:
10 µl
Detection:
At 360 nm.

Results

Two methods were used to analyse which flavonoids are contained, HPTLC and HPLC. With the HPLC the flavonoids could be quantitatively determined. Figure 1 shows the HPTLC fingerprint of the macerates of the buds.

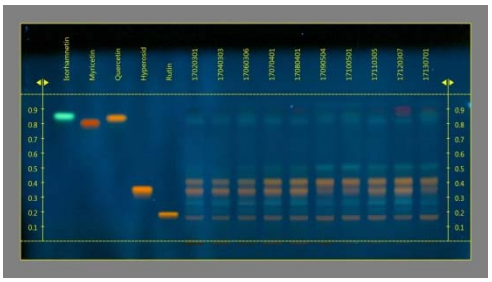

Fig. 1: HPTLC fingerprint of different batches of macerates of the buds.

➢ Identification of flavonoids

Hyperoside, isorhamnetin, myricetin, quercetin and rutin were used as reference substances for the HPTLC fingerprint. They all appear as fluorescent zones. The fingerprint of the test solutions, figure 1, shows the different batches. Only hyperoside and rutin could be identified clearly in all tested batches. The content of isorhamnetin is dubious. Myricetin and quercetin is definitely not contained. Due to these ambiguities an analysis with HPLC was made. The following phenols were studied:

- chlorogenic acid
- hyperoside
- isoquercitrin
- isorhamnetin
- isovitexin
- kaempferol
- luteolin
- quercitrin
- rutin
- vitexin

Figure 2 shows the quantitative evaluation of the concentrations of the phenolic compounds in the macerates.

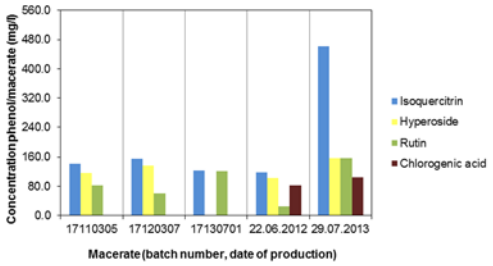

Fig. 2: Comparison of the phenolconcentrations in three macerates of buds and two macerates of leaves.

The flavonoids isoquercitrin, hyperoside and rutin were identified. Additionally contained in the macerates of the leaves is the chlorogenic acid.

➢ Homogeneity and stability of the macerates

Figure 1 demonstrates the fingerprints of different batches. The concentrations of flavonoids are similar in all analysed test solutions. With increasingly older date of production no decrease is seen.

➢ Comparison of the macerates of buds and leaves

There are some differences between the macerates of buds and leaves. Remarkable is that the macerate of leaves, named 29.07.2013, contains higher concentrations of all identified flavonoids (see figure 2). Exceptionally to mention is the high concentration of isoquercitrin, 461 mg/l.

Discussion

The flavonoids isoquercitrin, hyperoside and rutin are contained in the macerates. As well as other studies reported the identification of these flavonoids from black currants (He et al., 2011 & Vagiri et al., 2012). Although several studies confirm the presence of the flavonoids kaempferol, myricetin and quercetin (Vagiri et al., 2012), in this study they could not be identified. Further studies should be undertaken to clarify the inconsistent results.
In this study, the test solutions contained glycerol as opposed to the samples in the studies, which are described in the literature. Therefore there could be differences between the ingredients determined in this study and those which are described in the literature.
The results underline the fact that specific substances of the secondary metabolism as flavonoids can already be detected in the mesenchymatic tissue of the buds. Therefore possible effects of these substances which are known for leave extracts are also possible for bud extracts.

References:
He, D., Huang, Y., Ayupbek, A., Gu, D., Yang, Y., Aisa, H. A., & Ito, Y. (2011). Separation and Purification of Flavonoids from Black Currant Leaves by High-Speed Countercurrent Chromatography and Preparative HPLC. *National Institutes of Health Author Manuscript*, *33* (5), 615–628.

Ph. Eur. Monographie 1874, Hyperici herbae extractum siccum quantificatum.

Vagiri, M., Ekholm, A., Andersson, S. C., Johansson, E., & Rumpunen, K. (2012). An Optimized Method for Analysis of Phenolic Compounds in Buds, Leaves, and Fruits of Black Currant (*Ribes nigrum* L.). Journal of Agricultural and Food Chemistry, 60, 10501–10510.

Contact:
Spagyros AG, Tannackerstrasse 7, CH-3073 Gümligen

J. Ryffel, j.ryffel@spagyros.ch