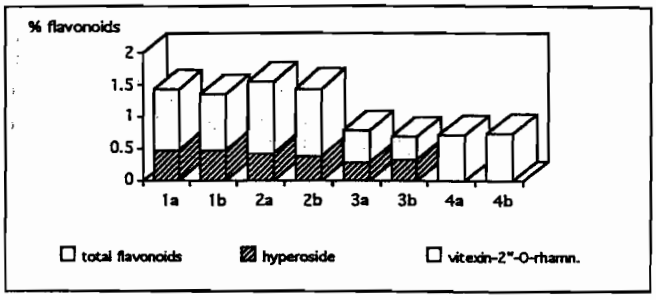

Fig. 1 Quantitative flavonoid determination of two samples a and b by four methods.

Therefore, a quantitative reversed-phase HPLC method was developed which is based on the reduction of the complex flavonoid glycoside pattern to a few major aglycones and *C*-glycosides by acid hydrolysis (2). The plant material was extracted in a Soxhlet apparatus with MeOH and hydrolyzed with hydrochloric acid under heating. The flavonoid *O*-glycosides such as hyperoside and rutin are hydrolyzed to quercetin, the main flavone *C*-glycosides vitexin 2″-rhamnoside and its acetate to vitexin. The determination of quercetin and vitexin was carried out with external standards on a Hypersil ODS 5 µm column. The mobile phase for quercetin was a gradient with MeOH and 0.5 % orthophosphoric acid. For vitexin a mixture of THF, isopropanol, acetonitrile, and 0.5 % orthophosphoric acid was used. Vitexin was calculated as vitexin 2″-rhamnoside and quercetin as hyperoside. Concerning the validation, the completeness of the extraction was tested. The content of flavonoids with a Soxhlet extraction was 15–20 % higher than with an extraction by reflux. The completeness of hydrolysis was checked by means of a fingerprint chromatogram. The derivatives of vitexin required stronger hydrolytic conditions than the flavonol *O*-glycosides, so these two groups were hydrolyzed and analyzed separately. The recovery was 96.4 % for vitexin 2″-rhamnoside and 91.5 % for hyperoside.

A second method utilizes a fingerprint chromatogram for the quantitative determination of vitexin 2″-rhamnoside and hyperoside. A methanolic extract was separated on a Hypersil ODS 5 µm column with a mixture of THF, acetonitrile, MeOH, and 0.5 % orthophosphoric acid as the mobile phase. Vitexin 2″-rhamnoside and hyperoside were determined with external standards. Acetylvitexin 2″-rhamnoside was calculated as vitexin 2″-rhamnoside. This chromatographic separation can be applied also for quality control.

Two commercial samples were quantified by four different methods. The results are shown in Fig. 1.

The hydrolysis and the fingerprint methods show similar results, while the method of Ph. Franç. X yields a clearly lower content of vitexin 2″-rhamnoside. The reason for this difference is, on the one hand, the incomplete extraction of the flavonoids and, on the other hand, the neglect of the acetylated vitexin 2″-rhamnoside which can be present, however, in a higher amount than vitexin 2″-rhamnoside, depending on the species (3).

We consider acid hydrolysis of the flavonoids to quercetin and vitexin as the nowadays most convenient method for the quantitative analysis of flavonoids in hawthorn as well as for the standardization of hawthorn extracts.

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Semi-Quantitative Salicin Analysis According the German Pharmacopoeia (DAB X): Avoidance of Picein Interference

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The phenolic glycoside salicin is of widespread occurrence in the genus *Salix* Linné (Salicaceae) and has been used in medicine for the treatment of acute rheumatism (1, 2). The quality of analgesic herbal remedies containing vegetable drug preparations can be determined by the total amount of salicin after alkaline hydrolysis (3, 4).

The DAB X monograph on Salicis cortex (5) describes a quantitative evaluation of salicin after alkaline hydrolysis and separation by TLC on silica gel G plates with ethyl acetate-formic acid-water (80 : 13 : 7). This determination is based on the comparison of the colour intensity of the standard and sample salicin spots after visualization with sulfuric acid-thymol. Unfortunately, this mobile phase is not able to separate salicin from picein, another phenolic glycoside of some willow species. Moreover, the reagent used for the detection gives the same violet colour for these two phenolic compounds. If the sample contains picein the results will not be accurate because the intensity of the spot will be a cumulation of salicin and picein spots.

For an unambiguous determination of the amount of salicin, a double migration on HPTLC silica gel 60 plates with ethyl acetate-methyl ethyl ketone-formic acid-water in a ratio of 60 : 20 : 2 : 2 (6) [slight but necessary modification of the mobile phase described in (1)] should be used in an unsaturated chamber. After separation, the derivatization should be achieved with the vanillin reagent described by Meier et al. (4): salicin appears as a violet spot and picein as an orange spot. By combining the use of both an adequate mobile phase and a suitable reagent, the examination of the plate will be more precise.

In the future, this semi-quantitative method based on the visual comparison of colour intensity of the spots could be replaced by validated instrumental techniques such as HPTLC densitometry (6) or HPLC (3). These methods give similar results and allow the quantitative evaluation of salicin after measurement of the absorption at 270 nm.

References

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