Characteristics of whole fresh plant extracts

Part 1: Results of analytical investigations

Methodology

Manufacture of tinctures

About 2 kg of freshly picked plants are divided into two equal parts. One part is dried in a drying cabinet for 24 hours at a temperature of 40 °C. The other part is processed immediately. Both the fresh and dried plants are cut with the Mado-Blitz Model MTK 50 (1 cm) and mixed with an appropriate amount of ethanol 96% V/V and water. Production is effected analogously with the manufacturing instructions contained in the HAB 1 (German Homeopathic Pharmacopoeia), paragraph 2a or 3a for fresh plants and paragraph 4a for dried plants. The ratio of plant to extraction medium (solvent) is, for both tinctures, approximately 1:10. The mixtures, contained in brown glass bottles, are stirred for 10 minutes 5 times a day for a period of 12 days (Janke and Kunkel RW 10 DZM). After this time, they are pressed (Hafico Model 5 l capacity, 450 bar) and filtered through a folded filter.

Echinaceae purpureae herba

Sample preparation

5.0 g of the tincture are mixed with 5.0 ml of the internal standard (4.0 mg propyl-4-hydroxybenzoate/100 ml ethanol 20% V/V), and diluted to 100.0 ml with ethanol 20% V/V. Approximately 5 ml of this solution are passed through a membrane filter (0.45 µm).

HPLC conditions

The caffeic acid derivatives and alkylamides are separated on a Lichrosorb RP18 column, 7 µm, 250 × 4 mm (Merck). The mobile phase employed is acetonitrile (solvent A) and water/phosphoric acid 100:1 (solvent B). The linear gradient starts at 15% A, increases, at a flow rate of 1.0 ml/min, to 80% A within 30 minutes, and remains at 80% A for 5 minutes. 20 µl amounts are injected; detection is effected at 254 nm. The content is computed with the aid of the response factors (caffeic acid 2.79; dodeca-2E, 4E, 8Z, 10 E/Z-tetraene acid isobutylamide 2.72). Cichoric acid is calculated as caffeic acid.

Crataegi fructus

Procyanidine and phenol content

2.0 ml of the tincture are made up to 100.0 ml with methanol 10% V/V (= stock solution). For determination of the procyanidines, 5.0 ml of the stock solution are applied to an RP18 cartridge (Baker 7020-03).
washed with 3 ml methanol 10% V/V, eluted with 6 ml methanol 50% V/V in a 10 ml measuring flask, and finally made up to the mark with methanol 50% V/V. To determine total phenols, 5.0 ml of the stock solution are diluted to 10.0 ml with methanol 50% V/V.

For the photometric measurement, 2.0 ml of the solution under investigation are mixed with 10 ml ethanol 25% V/V, 1% folin-ciocalteu reagent (Fluka), 10 ml sodium carbonate 70%, and diluted to 25.0 ml with water. After 25 minutes, the extinction of the centrifuged solution is measured at 750 nm against a blank solution prepared with 2.0 ml methanol. The content is expressed, calculated on the basis of the molar extinction coefficient (1250), as epicatechin.

**Procyanidine content (stability)**

In place of the RP18 cartridge, the procyanidines are separated on a glass column packed with 2.5 g of polyamide. The flavonoids are separated with 3 x 25 ethanol 50% V/V, and the procyanidines eluted with 3 x 25 ml dimethylformamide. The photometric measurement is again carried out with the folin-ciocalteu reagent.

**Symphytii officinalis radix**

**Sample preparation**

*a) Allantoin (HPLC)*

5.0 g of the tincture are diluted to 100.0 ml with ethanol 96% V/V and filtered through a folded filter. Approximately 5 ml are filtered through a membrane filter (0.45 μm).

*b) Pyrrolizidine alkaloids (densiometry)*

10.0 g of the tincture are processed using the method described by U. Zweifel and J. Lüthy [1].

**Conditions**

*a) HPLC*

The separation of allantoin is effected on a Lichrosorb RP18 column, 7 μm, 250 x 4 mm (Merck). The mobile phase is acetonitrile/water 90:10 at a flow of 1 ml/min. 20 μl amounts are injected. Detection is effected at 200 nm. The content is computed against the external standard (1.25 mg allantoin 100 ml ethanol 96% V/V).

**b) Densitometry**

The pyrrolizidine alkaloids are determined in accordance with the method described by Zweifel/Lüthy [1] using the CAMAG TLC scanner II. The content is computed against the external standard (10.0 mg seneciphylline/25 ml methanol).

**c) Viscosity**

The viscosity is determined at 20°C with the capillary viscosimeter in accordance with the European Pharmacopoeia II.

**Valerianae officinalis radix**

**Sample preparation**

*a) Valerene acid (HPLC)*

5.0 ml of the tincture are mixed with 2.0 ml of the internal standard (50.0 mg biphenyl/100 ml ethanol 96% V/V) and made up to 50.0 ml with ethanol 50% V/V. Approximately 5 ml are filtered through a membrane filter (0.45 μm).

*b) Essential oils (GC)*

10.0 g of the tincture are mixed with 50 ml water and 2.0 ml internal standard (50.0 mg thymol/100 ml ethanol 96% V/V) in a separating funnel. Extraction is then effected three times with 10 ml hexane. The pooled hexane phases are centrifuged and serve as the solution to be investigated.

**Conditions**

*a) HPLC*

The separation of the valerene acid derivatives is effected on a Lichrosorb RP18 column, 7 μm, 250 x 4 mm (Merck). The mobile phase employed is methanol/acetonitrile 40:60 (solvent A) and water/phosphoric acid 100:1 (solvent B). The linear gradient begins at 60% A, increases at a flow rate of 1.0 ml/min. to 80% A within 10 minutes, and remains at 80% A for 5 minutes. 20 μl amounts are injected. Detection is effected at 225 nm. The content is computed with the aid of the response factors (acetoxyvaleranic acid 0.72, valerenic acid 0.57, valerenal 0.53) indicated in the literature (2).

*b) GC*

Separation of the essential oils is effected on a Carbowax CW 20 capillary column (l = 25 m, diam. = 0.32 mm, df = 0.25 μm) from Macherey + Nagel. The carrier gas is hydrogen at a pressure of 0.4 bar (t = 0.8 min). The temperature gradient starts at 50°C, remains at this level for 5 min. and then increases immediately to 100°C, and finally increases, at 4°C/min., to 200°C, at which level it remains for 10 minutes. Amounts of 1 μl are injected with the PTV injector (50-150°C, 5 s) at a split of 1:27. Detection is effected with an FID at 250°C. The calculated content (sum of all peaks) is expressed as thymol.

**Achilleae millefolii herba**

**Sample preparation**

10.0 g of the tincture, together with 2.0 ml of the internal standard (thymol) and 50 ml water are mixed in a separating funnel. This mixture is shaken out four times with hexane. The pooled hexane phases are centrifuged and serve as the solution to be investigated.

**GC conditions**

The essential oils are separated on an SE-54 capillary column (l = 25 m, diameter = 0.32 mm, df = 0.25 μm) from Macherey + Nagel. The carrier gas employed is hydrogen at a pressure of 0.4 bar (t = 0.7 min). The temperature gradient begins at 50°C and then increases, at a rate of 5°C/min. to 200°C; thereafter, it increases immediately to 240°C, at which level it remains for 10 minutes. Amounts of 1 μl are injected with the PTV injector (50-200°C, 5 s) at a split of 1:27. Detection is effected with the FID at 250°C. The calculated content (sum of all peaks) is expressed as thymol.
Hyperici perforati
summitatibus cum floribus

Sample preparation
a) HPLC
2.0 ml of the tincture are mixed with 2.0 ml of the internal standard (15.0 mg xanthone/50.0 ml methanol) and made up to 50.0 ml with methanol 50% V/V. Approximately 5 ml of this solution are filtered through a membrane filter (0.45 µm).

b) Spectroscopy
5.0 ml of the tincture are made up to 50.0 ml with methanol. 5.0 ml of this solution are diluted to 20.0 ml and filtered through a membrane filter (0.45 µm).

HPLC conditions
The separation of the flavonoid glycosides, aglycones and hypericin derivatives is effected on a Supersphere 100 RP18 column, 4 µm, 250 x 4 mm (Merck). The mobile phase employed is acetonitrile/methanol/tetrahydrofuran/phosphoric acid 50:30:20:1 (solvent A) and water/phosphoric acid 100:1 (solvent B). The linear gradient begins at 20% A, increases, at a flow of 0.8 ml/min., to 100% A in 30 minutes, and remains at 100% A for 5 minutes. Amounts of 20 µl are injected. Detection is effected at 320 nm. The content is calculated with the aid of the response factors, which are determined previously against rutinoside (1.18), quercetin 2 H₂O (0.84) and hypericin (0.44). The flavonoid glycosides (rutinoside, hyperoside, isorhamnetin, quercitrin and two unknown flavonoids), are computed as rutinoside, the aglycones (quercetin, bisapigenin) as quercetin 2 H₂O, and the dianthrone derivatives (pseudohypericin, hypericin) as hypericin.

Spectroscopy
Measurement of the sample solution is effected against methanol using a 1 cm cuvette. Each measurement covers the spectrum between 400 to 700 nm, and absorption is determined at 590 nm by employing a tangent (630, 520 nm). The calculation is carried out using the specific absorption coefficient of 718 [3].

Betulae pendulae folium
Sample preparation
2.0 g of the tincture are diluted to 100.0 ml with methanol 20 V/V. Approximately 5 ml are filtered through a membrane filter (0.45 µm).

HPLC conditions
Separation of the flavonoids is effected on a Lichrosorb RP 18 column, 7 µm, 250 x 4 mm (Merck). The mobile phase used is acetonitrile (solvent A) and water/phosphoric acid 100:1 (solvent B). The linear gradient begins at 15% A, increases, at a flow of 1 ml/min., to 50% A within 30 minutes, and remains at 50% A for 5 minutes. Amounts of 20 µl are injected. Detection is effected at 360 nm. The content is computed against the external standard (hyperoside).

Cynarae scolymi herba

Bitter value
Determination is effected with DAB 10 (German Pharmacopoeia).

Cynaropicrine content
45.0 g of the tincture are determined titrimetrically using the method described by Schneider and Thiele [4]. The bitter value is computed as cynaropicrine.

Results of the analytical comparisons

Echinaceaee purpureae herba
As can be seen in Figure 1, the fresh plant product made from Echinaceaee purpureae herba contains approximately three times the amount of alkylamides than the product made from dried plants. The immunostimulatory effect of these products is presumably due in large measure to the alkylamides [5]. In this connection, consideration is also given to the cicoric acid, which both products contain in about equal concentrations. Since the alkylamides are relatively volatile, a large percentage is lost when the fresh plants are dried.

Crataegi fructus
Both products contain similar concentrations of oligomeric procyanidines. These are, however, stable only in fresh plant products (Figures 2, 3). Stability is an important feature of quality, and in the last resort, also a measure of the consistent action of the preparation. Comprehensive pharmacological and clinical investigation show that the oligomeric procyani-

Figure 1. Fresh plant/dried plant comparison for Echinaceaee purpureae herba (in Echinaforce)
dines and flavonoids are responsible for the positive effect on the failing heart [6].

An explanation for the decrease in procyanidine concentration in the three investigated extracts obtained from dried berries and an extract made from frozen berries has not yet been found. The three extracts obtained from fresh berries are all stable.

**Symphytum officinale radix**

The allantoin content and the viscosity (mucous content -> polyfructosanes) are about three times as high in the fresh plant product (Figure 4). Allantoin promotes wound healing and accelerates cell regeneration. The mucoid substances have a soothing effect on local irritation [7]. The undesired pyrrolizidine alkaloids are also extracted in larger quantities from the roots of the fresh plant. These are, however, removed from the final product.

In this example, extraction of the larger molecules through the intact cell walls of the fresh root takes place very rapidly and efficiently.

**Valeriana officinalis radix**

The product made from dried roots contains only about two-thirds as much of the essential oils and
valeric acid found in the fresh plant product (Figure 5).

In the case of the valerian root, however, the question as to what constitutes the active principle has not yet been clarified. In addition to the sesquiterpenes and valepotriates (which are stable), the essential oil is probably responsible for the effect obtained [8].

**Achilleae millefolii herba**

The fresh plant products obtained from the three types of yarrow contain approximately 20% more essential oil (Figure 6). The positive effect of yarrow on anorexia and dyspeptic complaints is attributed in particular to the essential oil.

**Hyperici perforati summitates cum floribus**

- **Ethanolic extract**
  The content of hypericin derivatives (dianthriones) and flavonoid aglycones is comparable. The product made from dried flower heads contains about 20% more flavonoid glycosides (Figure 7).

- **Oily extract**
  Lipophilic hypericin-like substances are detectable only in the fresh plant product.

In mild forms of neurotic depression, apart from the flavonoids, the hypericins are considered to be largely responsible for the positive effect [9].

Of the substances observed by us, only the flavonoid glycosides have been shown in most fresh plant/dried plant comparisons to be present in equal concentrations in the two products, or possibly in higher concentration in the dried plant product. The question as to whether enzymatic splitting off of sugars during the processing of fresh plants takes place, needs to be investigated for the specific plants.

**Betulae pendulae folium**

The known diuretic effect is certainly attributable to the flavonoids.
Valeriana officinalis L. s. str.
Common Valerian

Bornyl acetate

Valerenal: \( R^1 = \text{CHO} \quad R^2 = \text{H} \)
Valerenic acid: \( R^1 = \text{COOH} \quad R^2 = \text{H} \)
Acetoxvalerenic acid: \( R^1 = \text{COOH} \quad R^2 = \text{OCOCOCH}_3 \)

Achillea millefolium L. s. str.
Yarrow

Chamazulene

Achillicine

Hypericum perforatum L.
Perforate St. John’s Wort

Hyperoside

Hypericin

Quercetin
In this area both products are equivalent.

*Cynarae scolymi herba*

Both the cynaropicrin content (bitter substances) and the biologically determined bitter value (European Pharmacopoeia) are approximately twice as high in the fresh plant product (Figure 8).

The major active ingredients with an effect on dyspeptic complaints are the bitter principles. By acting on the taste receptors, these bring about a reflexory stimulation of salivary and gastric juice secretion.

*Chamomillae recutitae flos*

The comparison between the fresh plant and the dried plant was made by R. Carle [10] as follows:

Stability, measured over a period of 10 months, shows that in the case of the extract obtained from dried flower heads, a 70% decrease in matricine occurs. In contrast, no decrease in concentration is seen with the fresh plant (Figure 9).

The instability of the extract obtained from dried flowers cannot be explained by oxidative degradation, since the instability is also present in an inert gas atmosphere.

Additionally, drying of the flowers is associated with a loss of matricine of approximately 50%, and of bisabolol of approximately 30%.

R. Della Loggia et al. were able to show that the anti-inflammatory action of the fresh plant extract is about twice as powerful as that of the extract made from dried flowers. This may be explained by the matricine content. For determination of the anti-inflammatory effect, the croton oil oedema test was employed [11].

**Discussion**

The results obtained show that direct extraction from freshly harvested plants is very gentle on the ingredients. When the plants are dried, an appreciable amount of the substances (not merely of the volatile ingredients) is lost. Drying of the plants usually has little influence on the nature and distribution of the ingredients, but does influence their quantities.

In terms of the concentrations of the active ingredients so far known (sometimes also in terms of stability), fresh plant products often prove to be superior. This is in accord with Alfred Vogel's experience to the effect that fresh plant preparations exert a superior effect as compared with those made from dried plants. In this connection, it is of interest to note that homeopathic mother tinctures are, whenever possible, obtained from fresh plants.

The superior stability of the pro-cyanidines in Crataegus extract, the higher solubility of the lipophilic hypericin-like substances in oil from St. John's Wort and the higher bitter value in the extract from Cynara, all obtained from fresh plants, cannot be definitively explained. It is possible that secondary substances, which are lacking in the dried plant product may be responsible for these effects. It has long been known that secondary substances, such as, for example, flavonoids, tannins, saponins, mucoid substances, etc. have an influence on the efficacy and solubility or bioavailability of phytopharmaceuticals (in both a positive and negative sense). Thus, for example, the effects of a cup of coffee differ from those of a corresponding amount of caffeine, and this also applies to an equal degree to atropine vis-à-vis an extract from Belladonna, and to sennoside vis-à-vis an extract from Senna, etc.

For the decision as to whether a remedy should be made from fresh or dried plants, a number of plant-specific aspects first need to be clarified.

- Are there any analytical differences between batches from different years?
- Does the drying or extraction process result in enzymatic or other changes?
- Is there any difference in stability between freshly picked plant and dried plant products?
Standardisation (adjustment to stipulated concentrations of ingredients) of extracts and proprietary medicines can be effected only in cases in which the active ingredients of the plant employed are already known. The secondary substances are left completely out of account although, from the therapeutic point of view, they must be considered to represent important supplementary substances to the active ingredient.

The analysis of a so-called marker substance (an ingredient that is typical for a given plant and present in adequate concentrations) is considered to be an aid to determining quality which, although informative in terms of pharmaceutical quality, usually says little or nothing about therapeutic quality. Similarly, fingerprint analyses and standardised (validated) manufacture procedures are also only unsatisfactory aids.

On the basis of these facts, standardisation of plant medicines ought also to take account of the secondary substances which, in medicinal plants, extracts, infusions and tinctures are very often the basis for the particular therapeutic spectrum of plant remedies [12].

A further difficulty that should not be forgotten is that the composition of a remedy can be changed by the production process while, on the other hand the galenic form of its presentation has an influence on its stability.

One way of improving this situation, and one which Bioforce has been applying for years, is described below.

**Controlled biological cultivation**

Standardisation of plant remedies should begin already with the starting material, the medicinal plant, which should be obtained from biological cultivation (thus reducing pollutants) rather than by the collection of wild plants. The Swiss monitoring organisation (VSBLG), which keeps our biological cultivation under constant surveillance, is officially recognised by the EU.

For the cultivation of growing fields on a production scale, a considerable number of growing and cultivation experiments are necessary. We have considerable knowledge in this area, even in the case of uncommon plants, for the cultivation of which no information is to be found in the relevant literature. Through the use of our own seeds, cultivation on carefully selected sites with suitable soil-related conditions (away from traffic and industry) and harvesting at a time of the year shown by years of observation to be optimal, a certain consistency in the concentration of active ingredients can be achieved over the years.

Standardisation of plant remedies is facilitated by ensuring controlled biological cultivation (Table 1).

**Controlled and gentle production**

The flow chart (Figure 10) shows a comparison between products obtained from fresh and those from dried plants.

The production infrastructure is designed to ensure that the growing fields are as close to the production facility as possible. This makes it possible to extract the active ingredients from freshly harvested plants without delay, using ethyl alcohol. Losses of active ingredients are thus minimal, and the plants do not require disinfection. The extraction procedures are so optimal that the broadest possible spectrum of active ingredients of the plant in question is preserved.
<table>
<thead>
<tr>
<th>A. Vogel’s fresh plant products</th>
<th>Products made from dried plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selection, controllable</td>
<td>Dried plants, long transport distances</td>
</tr>
<tr>
<td>Pesticide-free optimal site</td>
<td>No or limited controllability</td>
</tr>
<tr>
<td>Controlable, optimal harvesting time plant health</td>
<td>From many sites in various countries</td>
</tr>
<tr>
<td>Controllable, rapid processing</td>
<td>Loss of volatile substances during drying. Disinfection by gasing, irradiation or pressure treatment with CO₂</td>
</tr>
<tr>
<td>With ethanol</td>
<td>Differing storage times, differing shelf life</td>
</tr>
<tr>
<td>Liquid products (phytotherapy/homoeopathy)</td>
<td>Needed to render active ingredients extractable</td>
</tr>
<tr>
<td>Very gentle, minimal loss of active ingredients</td>
<td>with methanol or denatured ethanol</td>
</tr>
<tr>
<td>Compression</td>
<td>Various methods</td>
</tr>
</tbody>
</table>

Figure 10. Comparison of manufacturing methods for products made from fresh plant as compared with dried plants
The manufacturing process (Figure 10) shows an environment-friendly production method (Table 2).

By pooling various tincture or extract batches to form homogenous large batches (e.g. mixing together individual batches to produce annual batches), fluctuations in active ingredient concentrations can additionally be smoothed out. The effectiveness of standardisation by pooling batches is shown by the example of Hypericum tincture (Figure 11). Here, 100 batches of Hypericum tincture (plants collected from the wild) were analysed over a period of 5 years.

The lightly and heavily hatched areas of the superimposed quality replica, shows the possible range of fluctuations in the groups of ingredients analysed. The larger the area, the greater the fluctuations in active ingredient concentrations. The lightly hatched area shows the possible batch-to-batch fluctuations in active ingredients. The relatively large fluctuations in the active substances from one individual batch to another illustrates the problem which is encountered when wild plants are used.

Since the remedial effect in humans very probably fluctuates in the same order of magnitude, the use of individual batches of Hypericum tincture (wild plants) would manifest wide fluctuations in quality and effect. By pooling approximately 20 batches of tincture to provide an annual batch (heavily hatched area), this fluctuation in quality can be greatly reduced.

The production flow chart clearly shows how quality control covers the entire process, from the seed via the plant, its harvesting and processing, right up to the finished product. In addition to every analytical step in the control process, those working in the gardens and in the production process also carry out comprehensive controls. Thus, for example, any impairment of plant quality (e.g. due to hailstorms or the action of insects, etc), can be more reliably identified by the experienced eye of the gardener than

- Use of the right species
- Origin of seed and plants known
- Cultivation conditions and harvesting times known
- Smaller fluctuations in concentrations of ingredients from batch to batch in comparison with plants collected from the wild or obtained on the dried plants market
- Relative concentration of the various ingredients always remain similar
- Less contamination with heavy metals and pesticides.

The strict regulations for foodstuffs (CH) and for maximum amounts of pesticides (D) can already be met at the plant stage (average lead and cadmium concentrations are 3 to 5 times lower than in plants obtained from the dried plants market).

Table 1. Standardisation factors associated with controlled biological cultivation

- Short transport distances for fresh plants
- No drying
- No disinfection needed
- No grinding needed
- No toxic extraction solvents used

Table 2. Environment-relevant factors of the production process

![Diagram of production process]

Figure 11. Hypericum perforatum 1986–1991: Standardisation by batch pooling
is possible by analysing the tincture. Since the entire developmental history of the product is known, its quality can be guaranteed.

**Standardisation** of plant medicines is supported by an optimal production infrastructure and monitoring of the manufacturing process (Table 3).

---

**Summary**

Standardisation based on only a single active ingredient or class of active substances is associated with the danger that the concentration of the secondary substances may fluctuate. The holistic approach to standardisation via controlled biological cultivation, pooling of plant-specific individual batches, and the rational control of production combine to guarantee a high level of consistency of the concentrations of all the active ingredients involved.

---

**References**


---

**Table 3.** Standardisation factors associated with optimal production infrastructure and manufacturing control

- Controllable harvesting
- Rapid extraction from the fresh plants as a result of short transport distances (disinfestation thus obviated, no loss of active substances)
- Pooling of single batches
- Complete history of the product from seed to medicine.

Thus, complete quality control and process validation are possible.

---

**Address for correspondence:**

Dipl. Chem. Martin Tobler (Chemist)
Bioforce AG
CH-9325 Roggwil TG