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Oil extracts of herbal drugs – optimisation of the extraction parameters

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The plant constituents of fourty two olive oil extracts from chamomile flowers [*Chamomilla recutita* (L.) Rauschert] were analysed by means of GC, VIS-spectrometry, and HPLC in order to assess the effectiveness of the traditional extraction methods of the German Homoeopathic Pharmacopoeia (HAB 2008). The influence of the extraction temperature and the extraction period as well as the influence of stirring during the extraction period and of a pre-treatment of the herbal drug with ethanol 94% on the extraction efficiency was also studied. The results are presented in the form of transfer ratios with regard to the essential oil, the carotenoids, coumarins, flavonoids and the phenolcarboxylic acids.

1. Introduction

Oil extracts of herbal drugs are commonly used in phytotherapy as therapeutic agents in ointments, creams and lotions and as ingredients in cosmetics such as massage or body lotions; in anthroposophic medicine they are applied for embrocations and oil baths. Four different regulations for the production of oil extracts from herbal drugs are established in the German Homoeopathic Pharmacopoeia (HAB 2008; regulation nos. 12d–g). They vary concerning the drug extract ratio (DER_{native}), the extraction temperature, the extraction period and the pre-treatment of the drug (Table). These regulations are the result of empirical knowledge and have never been experimentally examined. This paper is searching for the first time for the most efficient extraction parameters and methods with respect to a high content of plant constituents in the oil extracts. For this purpose the composition of 42 olive oil extracts which were produced by varying extraction temperatures (20 °C, 35 °C, 50 °C and 65 °C) and extraction periods (2 h, 4 h, 12 h, 24 h, 48 h, 168 h and 336 h) was analysed. The influence of permanent stirring during the extraction period and the pre-treatment of the herbal drug with ethanol 94 % were also studied. The result of this study might either confirm the traditional extraction methods of HAB 2008 as appropriate or stimulate the industry to modify their extraction methods.

Matricaria flower (Matricariae flos, Ph. Eur. 6.0; *Chamomilla recutita* (L.) Rauschert) was chosen as a representative example because of its content of well-investigated lipophilic and hydrophilic components namely essential oil, coumarins, carotenoids, phenolcarboxylic acids and flavonoids (Schilcher et al. 2005) whose transfer ratios from the herbal drug into the oil extracts were analysed. Quantification was completed by means of GC (essential oil), VIS-spectrometry (carotenoids) and HPLC (coumarins, phenolcarboxylic acids and flavonoids).

2. Investigations and results

2.1. Essential oil

The source chamomile contained 6.8 mL/kg of essential oil, the essential oil content of the oil extracts was calculated to amount from 4.8 to 6.3 mL/kg with reference to the herbal drug (drug extract ratio DER_{native} 1 : 10) corresponding to a transfer ratio of 70 to 92%. The main constituents of the essential oil monitored by means of GC which transfer from the drug into the oil were ß-farnesene, spathulenol, bisabololoxid B, bisabolonoxid A, α-bisabolol, chamazulene, bisabololoxid A and spiroether. Increasing temperatures and longer extraction periods improved the extraction efficiency (Fig. 1). Permanent stirring during the extraction and pre-treatment of the drug with ethanol increased the essential oil content of the oil extracts and accelerated the extraction, documented by the steeper curves within the first two days. Principal component analysis (PCA, not shown) of the compostition of the different oil extracts revealed that the pre-treatment of the drug with ethanol does influence the essential oil pattern mean-

Table: Externals of the HAB 2008

Regulations No.	Labeling	drug extract ratio (DER_{native})	Extraction temperature	Extraction duration	Pre-treatment
12d	H 10%	1 : 10 (drug : oil)	60–70 °C	4 h	94% ethanol
12e	H 5%	1 : 20 (drug : oil)	60–70 °C	4 h	94% ethanol
12f	W 10%	1 : 10 (drug : oil)	37 °C	7 d	–
12g	W 5%	1 : 20 (drug : oil)	37 °C	7 d	–

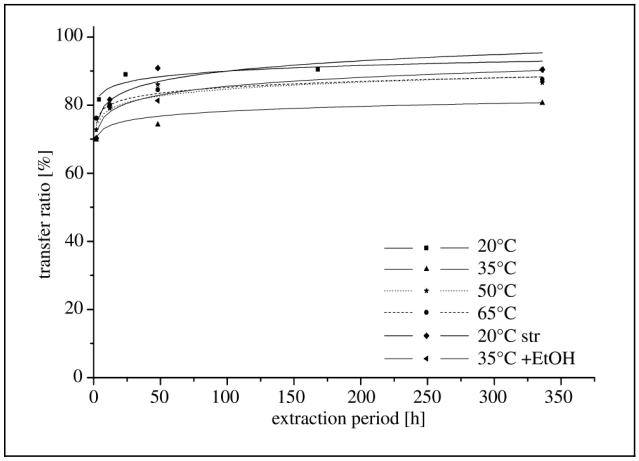

Fig. 1: Transfer ratio of the essential oil from the herbal drug into the oil extracts in correlation to the extraction parameters

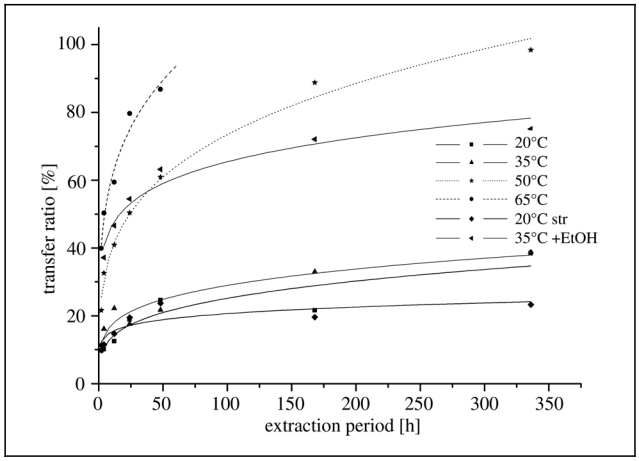

Fig. 3: Transfer ratios of the coumarins from the herbal drug into the oil extracts in correlation to the extraction parameters.

ing that the ethanol serves as a solubiliser for some of the oil components.

2.2. Carotenoids

The carotenoid content in the oil extracts ranged from 2.6 to 7.3 mg/kg and the content in the source chamomile was determined as 83.6 mg/kg. With reference to the herbal drug (DER_{native} 1 : 10) the transfer ratio runs from 31% to 87% (Fig. 2). Higher temperatures and longer extraction periods increase the carotenoid content of the oil extracts. Permanent stirring over a long period during extraction produced an abrasive dust, thus disturbing the VIS measurement. Therefore the two points of this curve at 7 and 14 days are not presented. Pre-treatment of the drug accelerated the extraction a bit but did not increase the carotenoid yield.

2.3. Phenolcarboxylic acids

HPLC analysis of the oily extracts showed that the phenolcarboxylic acids cannot be extracted from the herbal drug with the olive oil.

2.4. Coumarins

The coumarins in chamomile flowers are represented by herniarin und umbelliferone. The curves in Fig. 3 show transfer ratios from 10 to about 90% with increasing transfer ratios at higher temperatures and longer extraction periods as was found for essential oils and carotenoids. Permanent stirring over the extraction period was not efficient, but pre-treatment of the drug with ethanol raised the coumarin yield drastically.

2.5. Flavonoids

The transfer of the flavonoids is presented in three figures (Figs. 4–6) visualising the results of the individual groups of flavonoids (methoxylated flavonoid aglycones, not methoxylated flavonoid aglycones and acylated flavonoid glycosides). Again extraction at higher temperatures and for longer periods was more efficient. The highest transfer ratios were achieved for the methoxylated flavone aglycones, represented by chrysoeriol, isorhamnetin, chrysosplenol, jaceidin, eupalitin and chrysosplenitin, with values from 24 to 79% (Fig. 4). Permanent stirring during the extraction period increase the transfer ratio from 55 to 65% with a steeper increase in the first two days. Pre-treatment of the herbal drug with ethanol remarkably improved the yield of methoxylated flavones. The flavonoid aglycones (not methoxylated) luteolin, quercetin, apigenin and kaempferol showed the lowest transfer ratios (Fig. 5) with values below 10% at 20 °C and 35 °C. At 50 °C yields up to 32% were reached. Permanent stirring had no effect, but pre-treatment of the

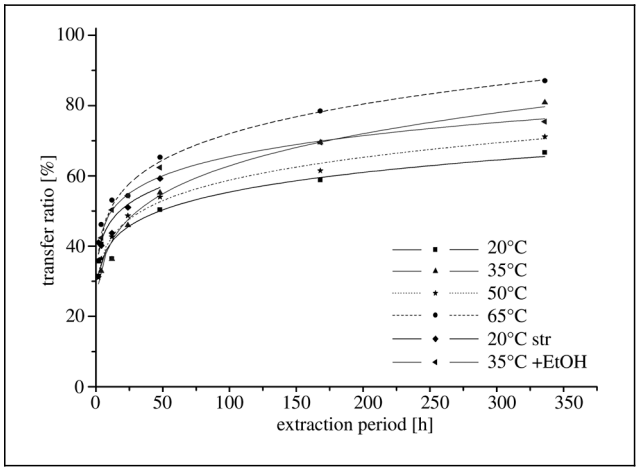

Fig. 2: Transfer ratio of the carotenoids from the herbal drug into the oil extracts in correlation to the extraction parameters

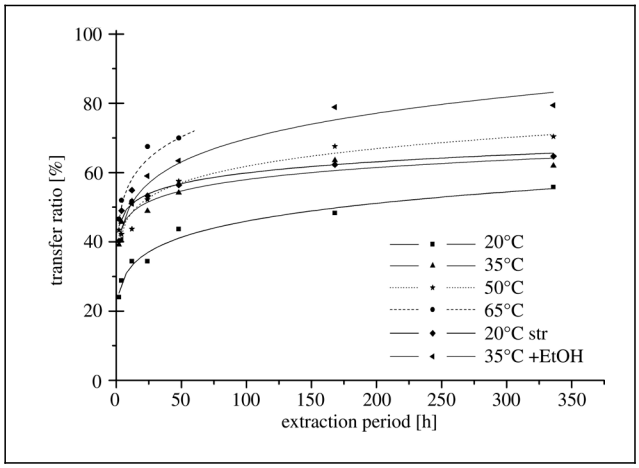

Fig. 4: Transfer ratios of the methoxylated flavone aglycones from the herbal drug into the oil extracts in correlation to the extraction parameters

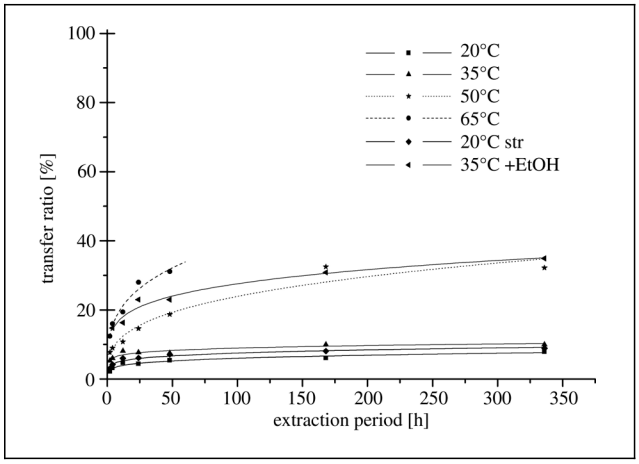

Fig. 5: Transfer ratios of the flavonoid aglycones (not methoxylated) from the herbal drug into the oil extracts in correlation to the extraction parameters

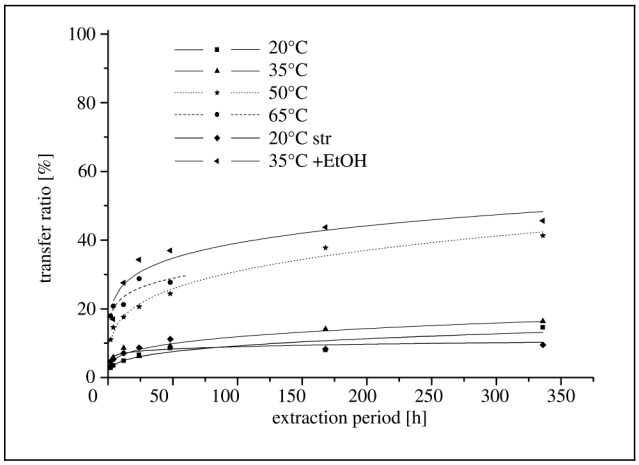

Fig. 6: Transfer ratios of the acylated flavone glycosides from the herbal drug into the oil extracts in correlation to the extraction parameters

herbal drug with ethanol increased the transfer ratio nearly fourfold, reaching about 35% (without pre-treatment 10%). The flavone glycosides are only extractable if the sugar moiety is acylated. The transfer ratios of the acylated flavone glycosides [apigenin-7-(4″-acetyl-glucoside) and apigenin-7-(-acetyl-malonyl-glucoside)] at 20 °C and 35 °C (Fig. 6) hardly exceeded 10% and permanent stirring had a positive effect at the first day only. Higher temperatures were more efficient, having transfer ratios up to 41% (at 50 °C). The same value was reached after two days at 65 °C.

3. Discussion

3.1. Influence of solubility und localisation

Discussing the transfer ratios of plant constituents into the oil extracts, the solubility of the components in the olive oil is the most important factor documented by the relative high transfer ratios of essential oils (up to 92%), carotenoids (up to 87%) and coumarins (up to 90%). The transfer ratios of the phenolic compounds consequently increase from flavonoid aglycones (up to 32%) over acylated flavonoid glycosides (up to 41%) to methoxylated flavonoid aglycones (up to 79%). Phenol glycosides (e.g. flavonoid glycosides) and phenolcarboxylic acids and their derivatives are not extractable from herbal drugs with olive oil. Earlier findings (Heldmaier and Stahl-Biskup 2006) showing that also the localisation of the plant constituents in the plant tissue or in the cells influences the transfer ratio of the plant constituents, were confirmed by the higher transfer ratios of the essential oil and the methoxylated flavonoid aglycones (surface of the epidermis) with those of the carotenoids located in the chromoplasts of the subepidermal layers.

3.2. Influence of extraction temperature and period

Figures 1–6 make obvious that higher temperatures as well as longer extraction periods result in higher yields of plant constituents in the oil extracts which can be explained by the higher penetration capacity of the oil caused by its lower viscosity at higher temperature (Basalo et al. 2006). However, the upper limit should be fixed at 50 °C or, in case of higher temperatures, the extraction period should be limited to 48 h in order to prevent the olive oil from becoming rancid which should be checked by the peroxide value. The steep rise of the curves within the first two days implies that a high portion of the plant constituents transfer within this period.

3.3. Influence of stirring

A positive effect of permanent stirring during the extraction period can only be observed for the essential oil and the methoxylated flavones which are located on the surface of the chamomile flowers (Repcak et al. 1999). The poor penetration of the oil through the cell walls obviously is a limiting factor (Basalo et al. 2006). Stirring over a longer period on the one hand has positive effects, on the other hand it causes disturbing abrasive dust after two days.

3.4. Influence of a pre-treatment of the herbal drug with ethanol

Ethanol obviously enables the oil to penetrate into deeper plant tissues, perhaps by destroying the cuticle, resulting in higher transfer ratios of all components. Moreover ethanol might act as a solubiliser. These effects have the highest impact on components that are localised deep in the plant tissue (e.g. coumarins) and on less fat-soluble components (e.g. flavonoid aglycones). However, changes of the component patterns of the oil extracts have to be considered (see essential oil).

3.5. Conclusion

Finally the results of the extractions of herbal drugs with oil are not always predictable because of the interacting lipophilic and hydrophilic components, the localisation of these components in the plant tissues and the oil matrix resp. and therefore individual testing is necessary.
Optimal results in the sense of a high content of plant constituents in the oil extracts can be achieved by two different extraction tactics: 1) low temperature (35 °C) over a long extraction period (at least one week); 2) high temperature (65 °C) for a short time (max. 2 days). Both procedures are more or less realised in the regulations of the German Homoeopathic Pharmacopoeia (HAB 2008). Permanent stirring is not very effective and sometimes disadvantageous causing abrasive dust which may cause problems with the products. Ethanol pre-treatment on the one hand increases the yield, on the other hand small alterations of the constituent pattern in the oil extracts must be considered.

4. Experimental

4.1. Plant material and preparation of the oil extracts

The oil extracts were produced in laboratory scale using chamomile [Matricaria flower, Matricariae flos, Ph. Eur. 6th ed.; *Chamomilla recutita* (L.) Rauschert; Caelo, Germany] and refined olive oil (Ph. Eur. 6th ed., Heess, Germany). Each extract was prepared with 1 part of herbal drug (50.0 g of Matricaria flower) and 10 parts of olive oil (500.0 g). DEV_{native} 1 : 10. The oil was separated from the herbal residuum in a pneumatic tincture-press (Hafico, Germany) and filtrated through a paper filter.

4.2. Isolation and determination of the essential oil

The essential oil of the source chamomile flowers (50.0 g) was isolated and quantified using a Clevenger-type apparatus according to the European Pharmacopoeia (Ph. Eur. 6th ed.; 4 h, 0.2 mL Xylol). The essential oil contents in the oil extracts were determined indirectly by hydro-distillation of the herbal residuum after extraction and calculated as the difference between the essential oil contents of the herbal drug itself and of the herbal residuum. The essential oil pattern of the oil extracts were analysed by GC after saponification (Heldmaier 2007).

4.3. Isolation and determination of the carotenoids

The total carotenoid content of the oil extracts was determined spectralphotometrically at a wavelength of 450 nm. The content was calculated by means of linear regression with a generated calibration curve of β-carotene (Heldmaier 2007).

4.4. Isolation and determination of the phenolic compounds

The phenolic compounds were isolated by liquid-liquid-partitioning according to Heldmaier and Stahl-Biskup 2006. HPLC-DAD-system and conditions see Heldmaier (2007).

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