

## Effect of Extraction Methods on the Composition of Essential Oils

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### Abstract

The aim of this work is to demonstrate our results on comparison of composition of essential oil fractions obtained by traditional steam distillation and supercritical fluid extraction. The plant materials for the various extraction methods were selected from the Asteraceae and Lamiaceae families. For the supercritical fluid extraction (SFE) carbon dioxide was used as supercritical solvent. The extracts were collected by stage wise precipitation in two separators. The waxy product and extract rich in essential oil were collected in the 1st and in the 2nd separator respectively. The traditional water steam distillation (SD) was carried out in the special apparatus of the Hungarian Pharmacopoea (7<sup>th</sup> ed.). GC analysis was carried out on capillary silica fused columns coated with DB-1701 and the specific chiral columns coated with Rt- $\beta$  DEX m or Rt- $\beta$  DEX sm. Comparing the composition of steam distilled oils with that of volatile SFE fractions the following general characteristics were established. The SFE fractions were richer in monoterpene-esters and poorer in alcohols than the traditional essential oils (clary sage, lavender). Regarding the distribution of the monoterpene and sesquiterpene compounds, the SFE fractions contained sesquiterpene hydrocarbons in higher percentage than the distilled oils (*Salvia triloba*). Furthermore, the proportion of sesquiterpenes increased in SFE fractions collected successively with time (*Salvia officinalis*) similar to the ratio of oxygenated monoterpenes to monoterpene hydrocarbons (*Rosmarinus officinalis*). In other cases it was verified that part of the mono- and sesquiterpenes were present originally in bound form (glycosides) in plants. Thus they appeared only in essential oil fractions after previous acidic treatment (*Thymus*, *Origanum*, *Satureja* species). During the supercritical extraction the azulenogene sesquiterpene lactones did not transform to azulenes (in chamomile, yarrow), but the non volatile SFE fraction of some Asteraceae plants contained sesquiterpene- $\gamma$ -lactones of unchanged structure.

### INTRODUCTION

Research on essential oils has been a permanent project in our institute for some decades including gas chromatographic evaluation and studying the influence of some technological and biological factors on their composition. The use of chiral gas chromatographic columns provided the possibility to investigate the stereospecificity of essential oil components.

The aim of this work is to present our results on comparison and composition of essential oil fractions obtained by traditional steam distillation (SD) and supercritical fluid extraction (SFE). The latter with carbon dioxide is a milder extraction method than the conventional steam distillation. This technique avoids the degradative heat processes, hydrolysis, isomerization and racemization. It is a special separation technique to obtain extracts and fractions of natural origin for therapeutical and cosmetic use (Chouchi et al., 1995; Dugo et al., 1995; Bicchi et al., 1999).

## MATERIAL AND METHODS

The plant materials used for this research were species from the Lamiaceae and Asteraceae families.

The plants were selected on the basis of the chemical structure characteristics of the main components of their essential oil: for example, monoterpenes: *Lavandula* species (lavander/lavandin), *Salvia sclarea* L. (clary sage), *Salvia officinalis* (sage), *Salvia triloba* L., *Rosmarinus officinalis* L. (rosemary), *Ocimum basilicum* L. (basil); phenolic compounds: *Origanum vulgare* L. (oregano marjoram), *Satureja hortensis* L. (savory), *Thymus serpyllum* L. (wild thyme); sesquiterpenes: *Chrysanthemum parthenium* (L.) Bernh. (feverfew), *Matricaria recutita* L. (chamomile), *Achillea millefolium* L. (yarrow), *Cnicus benedictus* L. (blessed thistle).

Extraction methods

**Steam distillation** from neutral and acidic media. The apparatus used, with a double condenser system, is official in the Hungarian Pharmacopoea (7th edition; Ph.Hg.VII., 1986). The weight of the starting crude drug was: 10-30 g.

**Supercritical extraction.** The apparatus developed by Technical University was used, which consisted of a storage vessel for the liquid CO<sub>2</sub>; the extractor and two separators. In the extractor, the fluid CO<sub>2</sub>, at a suitable pressure and temperature (300 bar, 40°C), came into contact with the ground plant material. The extract then passed through the pressure-reducing valves into the first separator (90 bar, 15-20°C) and then to the second separator (20 bar, 15-20°), where the fractionated separation took place by stagewise precipitation.

In the first separator a pasty oil extract (fixed oils, carotenoids, sterols, triterpenes) was deposited, while in the second separator a volatile oil rich extract was collected. The fractions collected successively with time provided the possibility to follow the extraction process.

GC methods for analysis of essential oil composition.

For the separation, identification and evaluation of volatile compounds, a gas chromatographic method was used. The GC analysis was carried out on packed and capillary columns respectively with nitrogen as carrier gas and a flame ionisation detector; the temperatures of detector and injector were 230°C and 200°C respectively.

A 3 m x 2.3 mm glass spiral column packed with 3% OV-17 and 30 m x 0.32 mm, silica fuse columns coated with DB-1701 of 0.25 µm, or specific chiral column coated with Rt-β DEXm and Rt-β DEXsm of 0.25 µm thickness were used. The columns were programmed as follows: 60°C-230°C, 8°C min., 230°C isotherm 3 min.

The oil components were identified by comparing their retention times with those of authentic standards, essential oils of known composition and peak enrichment. The confirmation of identity was done by comparison of their mass spectra with those reported in the literature (Stenhagen et al. 1974) and reference compounds.

## RESULTS AND DISCUSSION

For comparing the composition of essential oils obtained by traditional steam distillation (SD) and supercritical fluid extraction (SFE) the change of ratio in monoterpene-hydrocarbon enantiomer pairs; monoterpene-esters-alcohols; monoterpene hydrocarbons and oxygenated monoterpenes, as well as mono-sesquiterpenes was studied.

Essential oils rich in monoterpenes

Studying the ratio of the monoterpene *enantiomer pairs* in basil essential oils obtained by steam distillation (SD) and SFE it was found that in the case of three enantiomer pairs: camphene, β-pinene and limonene, the ratio turned over (Table 1).

The supercritical fractions were, in general, richer in *monoterpene-ester* components than the steam distilled oils. So, in the case of clary sage and lavender the ratio of linalyl acetate to linalool was higher in SFE fractions than that in SD oil. It was established that the high ratio of linalyl acetate to linalool was independent of the *Lavandula* plant-source, namely *L. officinalis* Chaix or *L. hybrid* Rox. L. (Lemberkovics

et al. 1998; Rónyai et al., 1999).

Both oils obtained from feverfew by SD and SFE contained two main components (camphor and chrysanthenyl acetate) but the percentage occurrence of chrysanthenyl acetate was higher in each SFE fraction. It was interesting that chrysanthenyl alcohol was not detectable in the oils after acidic steam distillation (Kéry et al., 1998).

Summing the percentage occurrence of volatile compounds of *Salvia triloba* according to their structure-type it was established that in SFE fractions the *monoterpene hydrocarbons*, *-alcohols*, *-ketones* and *-ethers* were present in lower amounts while *sesquiterpene hydrocarbons* and *oxygenated sesquiterpenes* were in higher quantities than those in distilled oil (SD) (Fig. 1).

Studying the change of ratio of *monoterpene hydrocarbons and oxygenated monoterpenes during the supercritical extraction* it was proved that the percentage values of monoterpene hydrocarbons decreased and those of oxygenated monoterpenes increased with time (Fig. 2). The data in the diagram are from five rosemary SFE fractions collected successively in time.

A similar tendency was observed with the *mono- and sesquiterpene hydrocarbon contents* of supercritical extracts of sage (Fig.3). The diagram shows the data of 3-3 fractions collected *successively in time* from two experimental runs.

Essential oils rich in phenolic compounds

The influence of extraction methods was studied in plants rich in volatile phenolic compounds such as oregano, thyme and savory.

In the case of oregano, the steam distillation was carried out under different conditions, such as: normal (neutral medium for 3 hours), long time (neutral medium for 6 hours) and acidic medium for 3 hours. In all cases, carvacrol was the main component, but two new components appeared in the long time and acidic water distillate (Lemberkovics et al., 1998).

The SD-oils of wild thyme were always richer in free alcohols and phenolic compounds (carvacrol, thymol) than the SFE fractions (Oszagyán et al., 1996).

In case of savory, steam distillation - both from neutral and acidic media - was carried out also from the residue of SFE. The main component was carvacrol in each fraction (similar to oregano and wild thyme); the distribution of other compounds is shown in Fig. 4. It was interesting that the percentage occurrence of  $\alpha$ -terpineol and an unknown alcoholic compound - which were present only in traces in SFE fractions - increased in the neutral and acidic steam distilled SFE residues.

The results proved that in these three carvacrol rich plants a part of the volatile compounds was originally present in bound form (as glycosides); and they were not extractable by fluid carbon dioxide.

Essential oils /plants rich in sesquiterpenes/ sesquiterpene lactones

Differences were found between the steam distilled oil and SFE fraction of chamomile and yarrow. Chamazulene, the main component of yarrow oil, was not detectable in the SFE fraction (Fig.5).

Probable the azulenogene sesquiterpene-lactones did not transform to azulenes during fluid extraction and remained in the plant matrix.

At the same time other, non-azulenogene sesquiterpene- $\gamma$ -lactones, such as parthenolide from feverfew and cnicin from blessed thistle, were detectable in the non-volatile part of SFE fractions (Kéry et al., 1999).

## CONCLUSIONS

- The essential oils obtained by supercritical fluid extraction are generally richer in ester constituents than the steam distillates.
- Changes in the percentage ratio in the SFE fractions collected during extraction successively in time is:
  - decrease of monoterpene hydrocarbons,
  - increase of oxygenated monoterpenes and sesquiterpenes.

- The essential oil components which occur in the plants in bound forms are either not present or are only in traces in SFE fractions.
- The azulene sesquiterpene lactones do not transform to azulenes during the fluid extraction but some non azulene sesquiterpene- $\gamma$ -lactones are extractable, with unchanged structure.

## ACKNOWLEDGEMENTS

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## Tables

Table 1. Ratio of enantiomer pairs <sup>(-)</sup>/<sub>(+)</sub> in Basil essential oils obtained by steam distillation (SD) and supercritical extraction (SFE)

Enantiomer pairs	SD	SFE
$\alpha$ -pinene <sup>(-)</sup> / <sub>(+)</sub>	4	8
camphene <sup>(+)</sup> / <sub>(-)</sub>	2	0.9
$\beta$ -pinene <sup>(+)</sup> / <sub>(-)</sub>	0,6	1,5
limonene <sup>(-)</sup> / <sub>(+)</sub>	16	0.5

## Figures

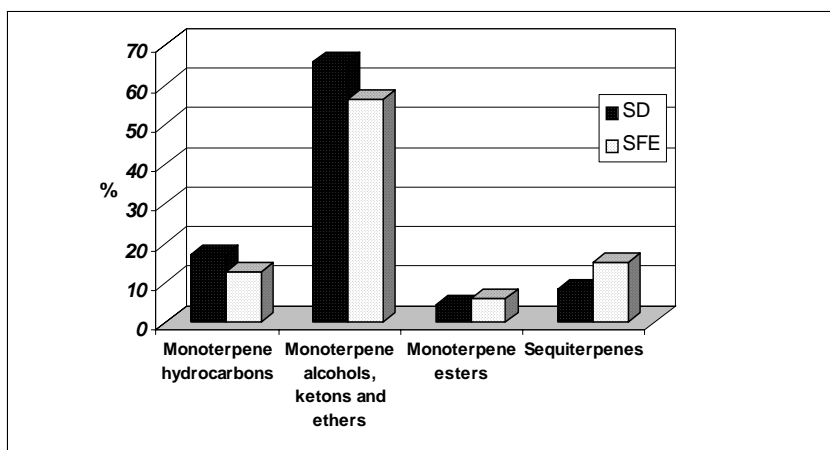


Fig.1. Characterisation of *Salvia triloba* oil composition obtained by steam distillation (SD) and supercritical extraction (SFE)

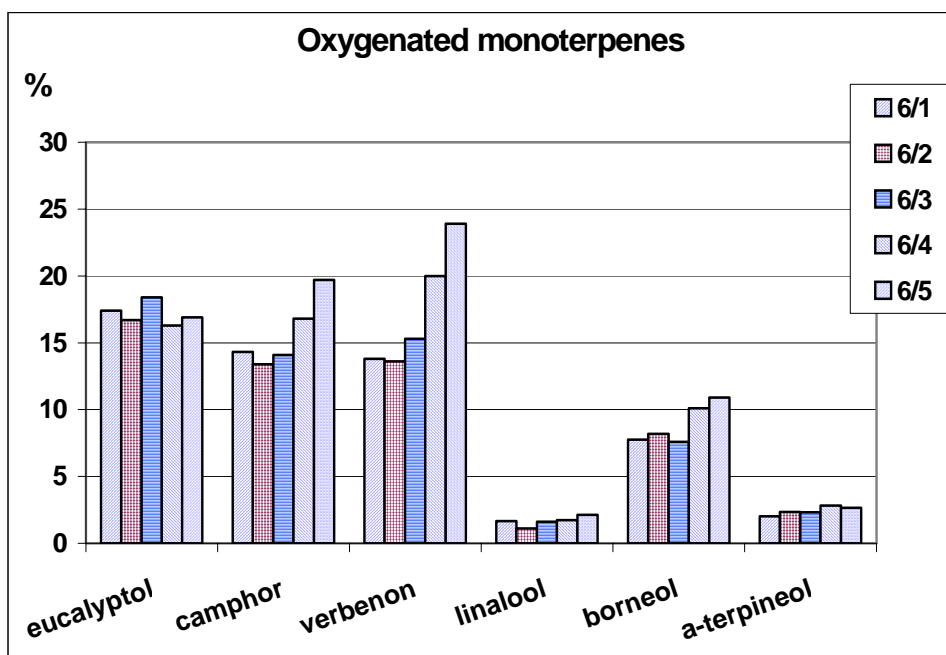
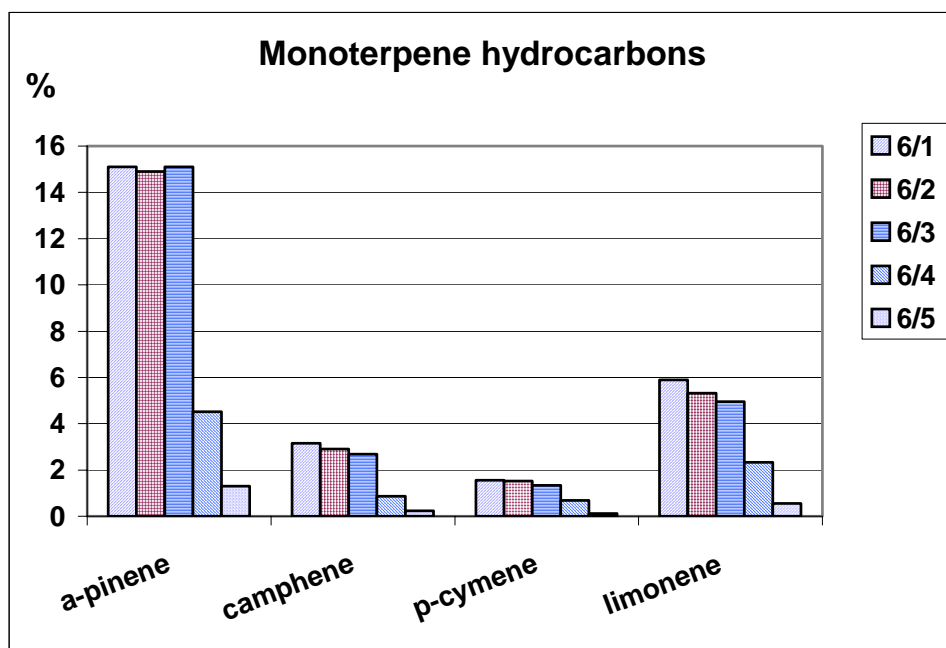


Fig.2. Change of monoterpene hydrocarbon and oxygenated monoterpene percentages (%) during the supercritical extraction of Rosemary (SFE fractions collected successively in time)

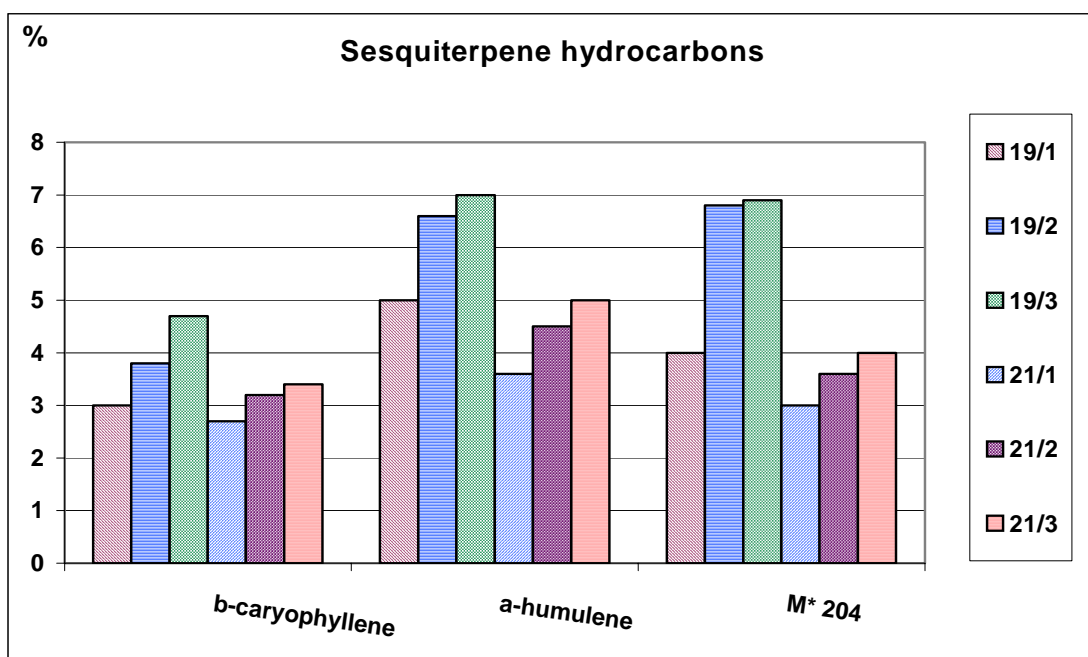
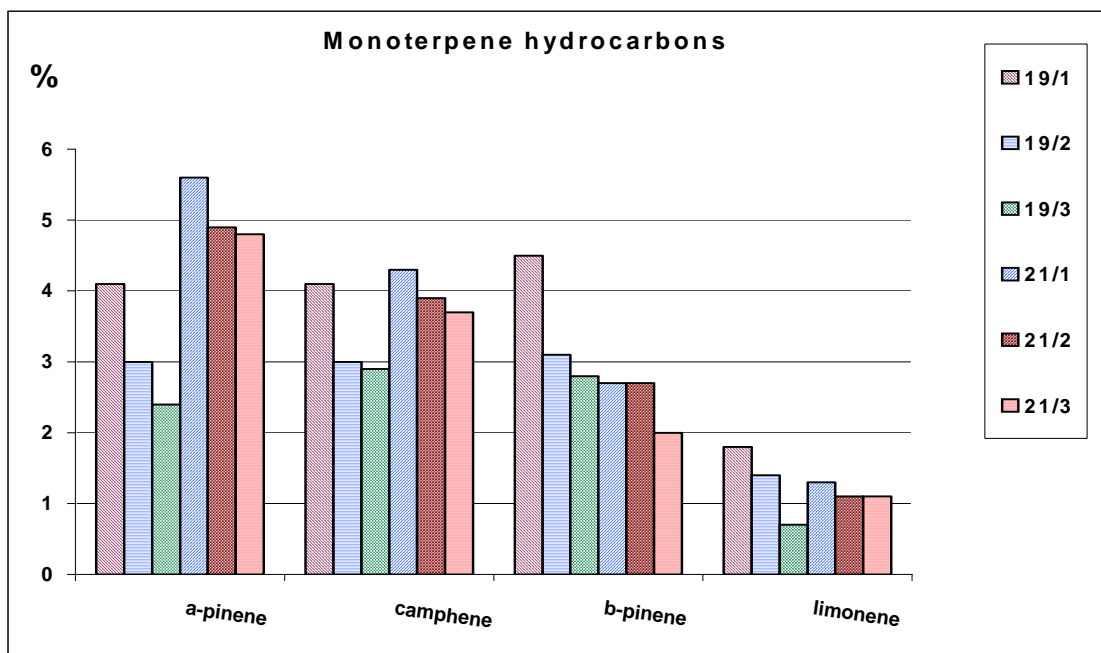


Fig.3. Change of mono-and sesquiterpene hydrocarbon contents (%) during the supercritical extraction of Sage (SFE fractions collected successively in time)

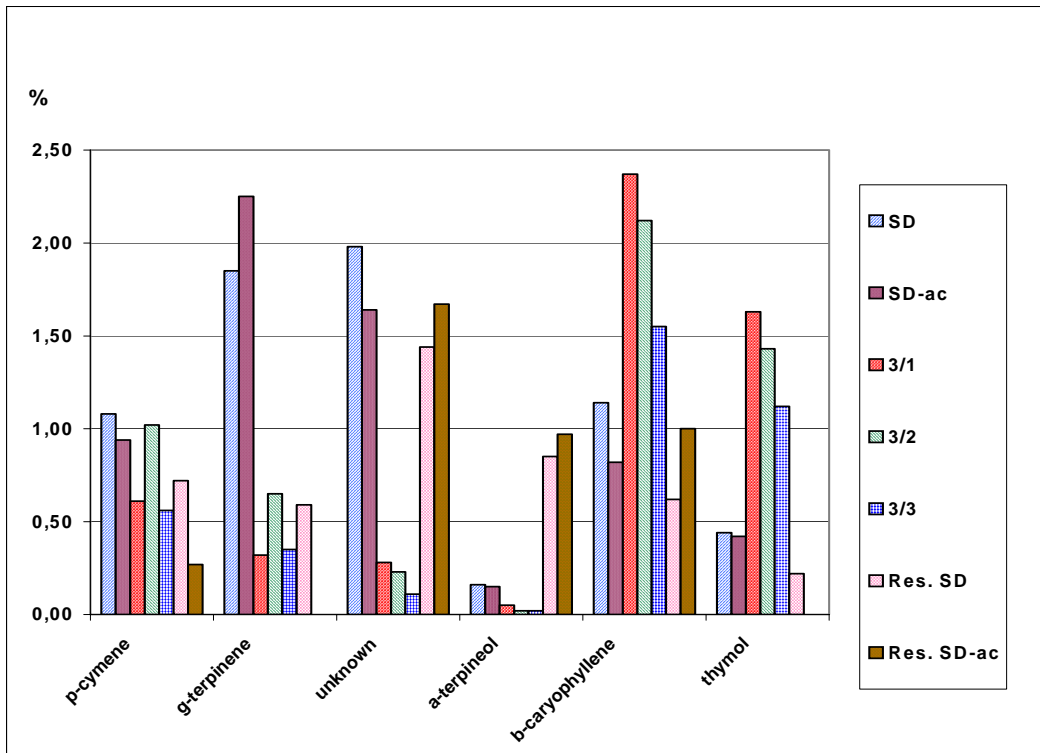


Fig. 4. Composition of essential oil and SFE fractions of *Satureja hortensis*