Phytochemical characterization of *Salvia*, *Lavandula* and *Morus* taxa by its terpene compounds

Ph.D. thesis

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1. INTRODUCTION AND OBJECTIVES

Gas chromatography plays a significant role in the analysis of essential oils, sterols and triterpenes, within which there is a need for further fast, reliable and precise-weaning techniques. Mass spectrometry is used for the identification of the components, while the most capable detection for percentage evaluation of terpenes is the flame ionization, caused by its greater sensitivity, and area normalization gives accurate results for essential oils. For the characterization of percentage distribution of the sterols and triterpenes the area normalization is indicative only, because mentioned components are less volatile and detection is not entirely quantitative, for its quantitation internal standard should be used.

Determination of the ratio of the volatile components present originally in plants is a difficult task, since the extraction method may affect the composition: during the water steam distillation, the high temperature and the aqueous medium may cause hydrolysis and izomerization. The essential oil composition, which closes the original fragrance of plants, can be determined by solid phase mikroextraction (SPME). Supercritical fluid extraction (SFE) is an increasingly popular method. The extraction is carried out by using fluid state inert gas, usually $C0_2$. The method is particularly suitable for extracting of non-polar compounds. The advantage of the method is that the solvent can be removed without a trace, by reducing the pressure of fluid gas.

The essential oil content and composition depends on both environmental and genetic factors. In some cases, beside the photochemical analysis, it is important to carry out molecular taxonomic study, too. Random amplified polymorphic DNA (RAPD) markers provide a convenient and rapid tool in assessing genetic differences between genotypes, even at lower intraspecific taxonomic levels. The patterns of relatedness observed in chemical profiles corresponded with the genetic profiles generated by RAPDs, suggesting that there may be a genetic basis for the chemical features.

That kind of model plants were chosen, in which the identification and quantitative measurement of the pharmacologically active compounds is an important practical task. The sage (*Salvia officinalis* L.) is one of the most important herbs in the Lamiaceae family; its drugs and its tincture are official in several pharmacopoeias (eg 8^{th} Hungarian Pharmacopoeia and 6^{th} European Pharmacopoeia). The major active ingredient of sage is its essential oil, which has antihypertensive, antimicrobial,

antispasmodic effects. The main component of the essential oil is thujone, through its neurotoxic effect; it should be used internally with care.

The true lavender (*Lavandula angustifolia* Mill, syn. *L. officinalis* L., *L. vera* DC.,) is also an important herb of the family. Its sedative, hypnotic, anxiolytic and antidepressant effect is due to its essential oil, in which the main components are the linalyl acetate and linalool. True Lavender Essential oil, and flower is offiinal at the 8th Hungarian Pharmacopoeia, and the 6th European Pharmacopoeia, and it also plays a significant role at the cosmetic industry.

The white mulberry (Morus alba L., Moraceae) is traditionally used in herbal remedies in the Far East; investigation of its leaf has yielded significant results in the therapy of diabetes. Recently, more dietary supplement containing mulberry leaf is marketed in Hungary. The anti-inflammatory activity of the sterol and triterpene molecules of *Morus alba* is based on the inhibition of the protein kinase-C enzyme.

The aim of our experimental work was to study the terpene compounds of *Salvia, Lavandula* taxa and *Morus alba*, using gas chromatography. Our plans included the comparison the essential oil content and distribution of the taxa described in the literature with taxa which has not previously been investigated or taxa designed from different continents as well. Our further aim was to carry out chemotaxonomic study of plants belonging to the same species, but having different phenotype The analysis of white and purple flowered, *'tricolor', 'purpurascens'* and *'Kew Gold' Salvia officinalis, S. judaica, S. africana-caerulea, S. mexicana*; furthermore *Lavandula vera, L. vera* ssp. *pyrenaica, L.intermedia, L. stoechas L.dentata* was planned. Our first aim was to identify the volatile components by GC-MS, determine the percentage composition by GC-FID, and study the impact of the extraction methods, such as solid-phase microextraction and water steam distillation for the essential oil composition. Our aim was also to observe the seasonal change of leaf oil of some taxa. In vitro cultivation of *S. africana-caerulea* for naturalisation purposes was also planned, such as producing viable open-planted plants, and testing its essential oil. We planned to observe the correspondence between the chemical profiles and the genetic profiles generated by RAPDs, suggesting that there may be a genetic basis for the chemical properties. Our further aim was to study the genetic background of the essential oil composition by RAPD markers and the percentage composition of essential oil, and then extend the

study of kinship more species of sage and lavender based on qualitative and quantitative analysis of the essential oil, by principal component analysis (PCA).

The aim of the examination of *Morus alba* leaf, and stem bark was to study their sterol and triterpene content, as well as to study the effects and optimize the conditions of the scale enlarging of the supercritical fluid extraction (SFE). Our goal was to examine the yields, unsaponifiable matter content, β-sitosterol content and terpene distribution of hexane, ethanolic extractions, as well as laboratory and pilot scale supercritical extraction. The quality assessment of phytosterols and triterpene components was designed by derivatization-free GC-MS method, β-sitosterol content planned to be determined by using an internal standard.

2. MATERIALS AND METHODS

2.1. Plant material

Leaf samples of sage taxa (white and purple flowered *Salvia officinalis, S. officinalis* cvs*. 'Purpurascens', 'Tricolor', and 'Kew Gold', and Salvia judaica,* 2007-2008.) and flowers of lavender taxa (*Lavandula vera, Lavandula intermedia, Lavandula pyrenaica*, and *Lavandula stoechas* ssp. *stoechas* 2008-2009) were collected in the Mediterranean collection of the botanic garden of the University of Pécs. *L. vera-1* and *L. intermedia* grown on limestone soils, the other sage and lavender plants grown on loess soils. The *Lavandula dentata* and *Salvia mexicana* plants originate from Los Angeles (California, USA, 2009); *Salvia africana-caerulea* plants (2008) come from the Kirstenbosch National Botanical Garden (South Africa, 2008). The *Salvia africana-caerulea* plants were propagated in vitro on MS medium (0.5% BAP and 2% sugar) and then planted in the ground, in a constant temperature room were grown, and then cultivated in the open field.

The Morus alba leaves and stem bark were collected in Budapest (South Buda, Gellért Hill), and Solt (Kiskunság) (2005-2006).

2.2. Extraction and purification methods

Essential Oil Extraction

Essential oils were obtained by water steam distillation for 3 h, using 30 g of dried, powdered plant material using the apparatus prescribed in the seventh Hungarian Pharmacopoeia, by using petroleum ether (in case of low essential oil content). Oil content was measured by volumetric or gravimetric method.

Solid-phase microextraction (SPME):

GERSTEL MPS SPME device, 0.3 g sample. Sample preparation: Incubator (60 ˚C, 5.00 minutes, shaking 250 rpm). Sampling: 65 μm PDMS / DVB (fused silica, STABLE FLEX / SS) adsorbent fibre (22.00 mm, 10.00 minutes). Injection: 44.00 mm, 60s, split injection (0.02 min, 50:1), heating: 250.0 ˚C, 43.0 mm.

DNA extraction (PTE Department of Plant Physiology)

Fresh leaves of sage, DNeasy Plant Mini Kit. The control of DNA content and the optimal dilution: by electrophoresis (1% agarose gel).

Organic solvent extraction in Soxhlet apparatus:

5g of powdered drugs in 100 ml n-hexane or 96 vol% ethanol, until the extract lost its colour. Cleaning: saponification (Ph.Hg.VIII.) Measurement: gravimetric.

Supercritical Fluid Extraction (SFE):

• Laboratory scale supercritical fluid extraction: 5 g of drugs, ISCO supercritical fluid extractor, fluid state carbon dioxide, a constant temperature (40 ° C), variable pressure: 100, 150, 200, 300, 400 bar, 60 - 90 min extraction time, CO_2 flow rate: 0.280-0.300 ml / min, sample purification: saponification (Ph.Hg.VIII.).

• Pilot scale supercritical fluid extraction (Budapest University of Technology, Department of Chemical Engineering): 1kg drugs, temperature: 40 ˚ C, CO2 flow rate of 7 kg / h, pressure 450 bar, two-stage decompression: 1 separator: 40 bar (herein after referred to as an extract used), 2. separator, 20 bar, extraction time: 510 minutes, Purification: saponification (Ph.Hg.VIII.).

2.3. Analytical methods

Thin Layer Chromatography (phytosterols, triterpenes preliminary examination): Layer: Kieselgel 60 F254. Standard: β-sitosterol 1 mg / ml hexane solution. Developing system: hexane - ethyl acetate 3:1, over a path of 10 cm, development: 0.5% cerium sulphate solution in sulphuric acid, evaluation: after heating (10 minutes at 100 ° C) in visible light.

Gas chromatography and mass spectrometry

- Analysis of the composition by GC-MS: Agilent 6890 N instrument, 5973N mass selective detector, Chrom Card Server Ver 1.2. program, 30 m x 0.25 mm column, stationary phase HP-5 MS, He carrier gas, injection: splitless. Column temperature for essential oils 1.: 60 ˚C, 3 minutes, 8 ˚C / min from 60 to 230 ˚C, 5 min 230 ˚C, essential oil 2.: 60 ˚C in 3 minutes, 8 ˚C / min, 60-200 ˚C, 200 º C 2 min, 10 ˚C / min from 200 to 250 ˚C 250 ˚C within 15 minutes, triterpenes test: 140 °C for 1 minute, 10 °C / min, 140-270 °C, 20 minutes 270 ˚C, 10 °C / min, 270-300 °C, 300 ˚ C 6perc. MS conditions: ionization energy 70eV, mass range 40-500m / z,. Identification of peaks: NIST spectral library and literary data. Percentage evaluation: RSD <7.25% 4 measurement.
- Analysis of the essential oil composition by GC-FID: Fisons 8000 gas chromatograph column: 30 m x 0.25 mm Rt-β-DEXm, carrier gas: nitrogen, injection: 0.2 to 0.4 ml of essential oil in chloroform (2µl/ml), splitless. Column 8 ˚C / min from 60 to 230 ˚C, 5 min 230 ˚C. Identification of peaks: retention time and standard addition; percentage evaluation: area normalization, 3 parallel measurements, RSD <4.5%.
- Analysis of sterols and triterpenes by GC-FID: Agilent 6890 N apparatus, column: 25 m x 0.2 mm, DB-5 MS stationary phase, carrier gas: He, injection: split. Column temperature: 120 °C for 1 min, 10 °C / min from 120 to 300 °C and 300 ˚C 14 min, 10 °C / min from 300 to 310 °C, 10 min 310 ˚C. Identification of the components: retention data, standard addition, percentage evaluation: area normalization, quantitative determination of β-sitosterol content: 5-α-cholestan-3-on internal standard. Three parallel measurements RSD% = 1.94%.

Molecular taxonomic studies

- RAPD (PCR) reaction: 62 decamer primer: OPA-(1-20), OPB-(2-20), OPN-(4), OPO-(1-19). Temperature program: 94 ˚C 2 min, 94 ˚C 10s, 36 ˚C 30s, 72 ˚C, repetition 35 times, 72 ˚ C 2 min
- Gel electrophoresis: 1.5% agarose gel, 110 V, 55mA, 2 hours, test: DNA Ladder +. Evaluation under UV light.

2.4. Statistical methods

- Cluster analysis based on RAPD fragments: a binary data matrix, Jaccard index; based on essential oil composition: continuous variables, Bray-Curtis coefficient, UPGMA, dendrogram (SYN-TAX 5.0 program).
- PCA study on the basis of the essential oils composition: qualitative analysis (Jaccard index), percentage evaluation (Bray-Curtis coefficient). Components responsible for differences were represented by Biplot analysis.
- Analysis of variance (one-way ANOVA) was used to test the significance of differences between means of data groups at $p < 0.05$. Each experiment was performed in triplicate.

3. NEW RESULTS AND CONCLUSIONS

3.1. Experimental results on *Salvia* taxa

Determination of essential oil content

- The highest essential oil content was gained from the leaves of *Salvia africana-caerulea* and *S. officinalis* cv. *Kew Gold*, which was approached by the white-flowered sage leaves. The purple-flowered, *tricolor* and *purpurascens S. officinalis* have moderate oil content, the *S. judaica* and *S. mexicana* contain volatile oil only in traces.

Qualitative (GC-MS) and quantitative (GC-FID) analysis of essential oil

In the essential oil of sage, 56 volatile components were identified. The essential oil composition of *S. officinalis* cv. *purpurascens, Kew Gold*, *tricolor*, and *S. Judaica* has been written firs time by us.

- The essential oil of the leaves of *S. officinalis* taxa contains the same components; the only difference is their ratio. To the leaf oil of white-flowered *Salvia officinalis*, a higher ratio of monoterpenes is characteristic; the main component is the toxic α-thujone. In the oil of the purple-flowered sage, and the ornamental cultivars, mono-and sequiterpenes occur in equal ratio: mainly humulene, α-and β pinene, 1,8-cineole and camphor.
- In judean, african and mexican sage oil the sequitqrpenes are dominants against monoterpenes, the greatest difference is expressed in the mexican sage oil (86:2). The main volatile components of the judean sage oil are β-cubebene, β-caryophyllene and ledol; of the african sage are piperitone and the β-copaene, and of the mexican sage are the β-caryophyllene, isoledene, β-copaene and farnesene.
- Diterpene epimanool occurs only in the oil of *S. judaica*, other typical sage diterpenes cannot be extracted by water steam distillation.
- Among the ornamental *S. officinalis*, in the *purpurascens* and *tricolor* variants, the hydrocarbons are predominant, mainly due to their α-humulene content. Oxygenated terpenes are present in the highest proportion in the white-flowered sage, they are mainly alcohols and ketones, but it also contains ethers and esters. Of the purple-flowered taxon, nearly 50% is the oxygen-containing mono-and sesquiterpene contents, while the ornamental variants, as well as the judean sage contains about 40%, the african and mexican sage only 6% of them.
- Comparing the flower and leaf essential oil composition of the white-flowered *S. officinalis* we found that α-thujone was as a significant component in the flower, as like in the leaf, but the ratio of sesquiterpenes was higher in the flower. The other major components are β-caryophyllene, α-humulene, ledol, viridiflorol, and ar-turmerone. The extraction of the lower thujone-containing oil is not economical, because the essential oil content of the flower was less than a half that of the leaf.

- There is a difference in oxygenisation between the leaf- and flower-oil, as well. Although the oxygenised terpenes are in the majority in both organs, the difference in the ratio of the two molecule groups is smaller in the flower. Moreover, the ratio of alcohols is higher in the flower, and the ratio of ketones is higher in the leaf due to the α-thujone content of the latter.

Comparison of the essential oil components of the ornamental variants of S. officinalis in different flowering periods

- The essential oil composition substantially changes in the flowering stage regarding the percentage of monoterpenes. This also includes the continuous rise in the neurotoxic α-thujone content during the flowering period, being the highest after flowering, in September. The sesquiterpene ratio is reduced accordingly, but the ratio of the main component; α-humulene is the highest during flowering.
- The ratio of oxygen-containing terpenes does not change significantly before and during flowering, but starts to rise in the autumn. The proportion of alcohols and ethers shows no clear change, the amount of ketones increases after flowering, while the ratio of esters is reduced.

The gas chromatographic studies revealed that the high essential oil content of the white-flowered sage leaf is coupled with a high neurotoxic thujone proportion; therefore that method is not very suitable for the safe extraction of essential oils in effect. Safe essential oil can be extracted most economically from the leaf of *'Kew Gold'* version collected during full flowering.

Essential oil analysis of *Salvia africana-caerulea* in vitro leaf cultures

The *Salvia africana-caerulea* leaf significally differs not only in its high essential oil content but also in its essential oil component from that of the *S. officinalis* with well-established medical effects. Therefore more studies are warranted in demonstrating the traditionally experienced biological effects of the essential oil.

Salvia africana-caerulea was derived from the collection of the Kirstenbosch National Botanical Garden (South Africa), so for future studies, as well as for domestic naturalisation we propagated this plant in tissue-culture for the first time, and successfully planted the viable plants in the ground. We compared the essential oil

content and composition of the original intact plants with the in vitro cultured ones and with the planted herbs.

- The essential oil production in intact plants has not been achieved in the in vitro cultures, while the essential oil content of the planted herb reached only one-third of the original plant.
- The essential oil composition does not change significantly. In each sample the sesquiterpene hydrocarbons are in the highest proportion, while the proportion of monoterpenes in vitro cultures and in planted herbs is about half of the intact plants.
- Among oxygenated terpenes the ketones dominate in all three samples, especially piperitone. Among sesquiterpenes the higher proportion of β-caryophyllene and γ-elemene are characteristics. These are the main components of essential oils in planted herbs. Optimising tissue culture conditions for *S. africana-caerulea* and demonstrating its phytopherapeutic effects will be the main goals of our future investigations.

The effect of extraction methods on the composition of volatile components

Salvia taxa were compared for the first time in their essential oil contents gained by water steam distillation and by SPME extracts. Since the SPME extraction is waterproof, and the temperature is lower, the components do not brake down, but the proportion of volatile components increases. Examining the effect of the extraction method exerted on the volatile oil components we have found the following:

- According to our expectations the higher proportion of smaller molecular weight and more volatile monoterpenes are characteristics of the SPME extracts, whereas sesquiterpenes dominate in water steam distillation.
- Oxygen-containing terpenes are always found at a higher rate in water steam distillates, the biggest difference is found in alcohols. Ethers of the SPME extracts are present in higher proportion, some of them are supposed to break down to alcohols during water steam distillation.

Molecular and chemotaxonomic studies

DNA-based molecular markers (RAPD) and the known composition of essential oils can be used to study the genetic determination of essential oil composition. We have

studied the first time the genetic and chemical variability of the above mentioned sage taxa. According to the genetic and essential oil composition computed on the basis of hierarchical cluster analysis we have found the followings:

- The closest relationship among *S. officinalis* taxa is found between the '*purpurascens'* and '*tricolor'* versions.
- Cluster analysis based on RAPD fragments and essential oil composition gives the same result, this refers to a close relationship between the chemical profile and the genetic pool. Our results confirm that the chemical profile and RAPD markers are both effectively applicable to examine relationships between different versions of sage.

The relational examination of sage essential oil composition was extended for each taxon on the basis of our study. We have performed PCA examination for the first time to illustrate relationships among a larger number of taxa, using vectors in a two-dimension coordinate system:

- *Salvia officinalis* taxa form one group, other taxa separate from those, and the more closely related taxa to the *S. officinalis* and to each other are *S. mexicana* and *S. africana-caerulea.*
- On the examination based on the presence of binary components, the purple-flowered version separated the most among *S. officinalis* taxa. The white-flowered version stands closest to the '*purpurascens'* version. According to the percentage composition of essential oils the purple-flowered *S. officinalis* forms part of ornamental variants, and shows closer relationship with the *'purpurascens*' and '*tricolor*' versions, than the *'Kew Gold'* taxon.

The relationship analysis was amended by biplot analysis, which illustrated the components responsible for the internal structure of the relationship, confirming our earlier findings.

• Ledol and viridiflorol are responsible for the separation of judean sage, whereas the distance between the african and mexican sage is caused by the co-existence of more components, namely β-caryophyllene, isoledene, γ-elemene, terpineol, γ-cadinene and piperitone.

• Among *S. officinalis* variants the relational distance of the white-flowered sage is clearly indicated by α-thujone, whereas the distance of the purple-flowered version is

caused by eucalyptol and to a lesser extent, γ-muurolene, α-humulene, α-thujone and camphor are the most responsible components for the difference of ornamental variants.

3.2. Experimental results on *Lavandula* taxa

Determination of essential oil content

- According to data of the literature the flower of *L. intermedia* has the highest essential oil content, but the volatile oil content of *L. vera* flower also exceeds the limit of the Ph.Hg.VIII. (min. 13 ml/kg). The essential oil content of *L. stoechas* is lower than the above mentioned ones, and the flower of *L. dentata* contains only traces of it.

Qualitative (GC-MS) and quantitative (GC-FID) analysis of essential oil composition

- We have identified 46 components in the essential oil of lavender taxa
- Linalool is the main component of the essential oil of the flower of *Lavandula vera* and *L. intermedia*, except for the oil of *Lavandula vera-3*, whose main component is lavandulyl acetate. The latter taxon has been first described by us. Other important components are linalyl acetate, camphor, and terpinene-4-ol, and eucalyptol for *L. vera* and *L. intermedia*. The ratio of linalool to linalyl acetate is a characteristic value of the different lavender species. The rate of linalool varies from 21 to 44%, being the highest in hybrid lavender oil. The ratio of linalyl acetate varies from 10 to 14%, reaching the highest value in the oil of Pyrenean lavender, so this taxon provides the most valuable lavender oil.
- The main component of the essential oil of *Lavandula stoechas* is fenchon, and its ester called fenchil acetate.
- The content and composition of the essential oil of *L. dentata* flower and leaf have been first compared by us. Interestingly, the flower contains only traces of oils; the oil content of the leaf is higher. The essential oil composition of the leaf and flower did not differ significantly. The main component of the essential oil is eucalyptol, reaching a higher percentage in the leaf, while it contains larger amounts of linalool, as well. The other main component of the oil of the flower, trans-pinocarveol can be

found in the leaf in lower proportion. The leaf's typical component is the β-pinene, as well. We can conclude that the essential oil can be extracted more economically from the leaf of *L. dentata* than from the flower.

- The higher amount of oxygen-containing monoterpenes is characteristic of lavender flower oils, with Pyrenean lavender having the highest monoterpene content, while the highest sesquiterpene content has the oil of the flower of *L. dentata*.
- The oil of *L. stoechas* has the highest proportion of oxygenated terpenes, while the largest hydrocarbon ratio can be measured in *L. vera-3* sample. The oxygen-containing components are mainly alcohols and esters with lower incidence of ethers and ketones. *L. vera-3, 4* samples contain the highest proportion of esters, alcohols dominate the oil of *L. intermedia*, in accordance with the data in the literature.

Effect of extraction methods on the composition of volatile components

The composition of essential oils derived by water steam distillation and solid-phase microextraction (SPME) has been first compared by us.

- On the basis of the volatility of mono- and sesquiterpenes based on their molecular weight we expected higher monoterpene ratio in SPME extracts, but the facts contradict to this hypothesis.
- The ratio of carbon and oxygen-containing terpenes varies significantly, the higher proportion of more volatile hydrocarbons is characteristic of the SPME extracts, in accordance with our expectations.
- Lavender essential oil is suitable for studying ester hydrolysis during water steam distillation. According to our expectations the ratio of esters is higher in SPME extracts than in distilled essential oils, the latter are richer in alcohols, however.

Chemotaxonomical studies

We have performed first a PCA test based on the binary and percentage composition of essential oils of lavender taxa.

- *L. vera* samples form a cluster: Pyrenean lavender is separated from the rest of the samples, the closest relationship can be detected between the samples of *L.vera1, 2*.
- According to the biplot analysis mainly fenchon is responsible for the separation of *L. stoechas*, and to a lesser extent camphor, and terpinen-4-ol. The more distant

relationship of *L. dentata* can be attributed in one hand to trans-pinocarveol, and on the other hand to the low content of linalool and linalyl-acetate. The differences among hybrid lavender, *L. vera-2*, and the Pyrenean lavender trace back to their linalool content. *L. vera* essential oils do not differ significantly in their linalyl acetate content, closest to its vector is situated that of the *L. vera-1* and of the Pyrenean lavender. Our PCA analysis demonstrated that Pyrenean lavender, which is often described as a separate species in literature, is forming a part of other medicinal lavender taxa, and is not a separate species, but a subspecies of *L. vera* instead.

3.3. Experimental results on *Morus alba*

The analysis of *Morus alba* leaf and bark di-and triterpene components complements the mono and sesquiterpene volatile compound analysis of *Salvia* and *Lavandula* species. The primary objective of the examination of the mulberry tree terpenoids was the escalation and optimalisation of the conditions of the SFE extracts. *The effects of the escalation on the yield and composition of extracts, and on the β-sitosterol content have been first described by us.*

Comparison of the yield of different extraction methods

Comparing the yield of different extraction methods with the crude extracts from non-saponifiable material (purified extract) content we have found the following:

- The ethanolic extract yielded in higher amounts than the hexane extract, but the amount of its unsaponifiable part is lower, which can be explained by the high yield and low selectivity of the ethanolic extraction.
- The analytical scale supercritical fluid extraction yield is very low. Applying various pressure and extraction times -in accordance with our expectations- the 400 bar pressure for 90 minutes has the highest extraction yield. The pilot scale supercritical fluid extraction yield is higher, but the yield is still only less than half of the hexane extraction. The unsaponifiable matter content of extracts does not show any significant difference.

- Extractions from the bark result in significantly higher yields in all cases than the leaf extracts, but the amount of the unsaponifiable part of the bark extracts is lower than that of the leaf extracts, which refers to a lower apolar content of the bark.

Identification of terpenes in Morus alba extracts

Comparing the qualitative assessment of phytosterol and triterpene components using thin layer chromatography with preliminary GC-MS examination we have concluded that:

- In *Morus* leaf extracts purified by saponification we have identified sterols: β-sitosterol and lanost-7-ene-3-on; triterpenes: α-amyrin, lupeol, and an open-chain diterpene called phytol; the latter is the main component of the leaf extracts. Lanost-7-en-3-on and lupeol components in Morus leaf and bark extracts were firs described by us.
- β-amyrin is also identifiable in purified extracts of bark, the ratio of the main component; α-amyrin reaches 50%.

Quantitative determination of β-sitosterol content

We found that pilot scale supercritical fluid extraction is the best method for extraction of β-sitosterol; its β-sitosterol content exceeds the content of the hexane extract.

- Among the analytical SFE extracts samples produced at 200 bar for 90 minutes, and at 300 bar for 60 minutes yielded in the highest β-sitosterol amount.
- The most suitable method for extraction of the bark is hexane-extraction and SFE with at 400 bar for 60 minutes. The β-sitosterol content of the bark is always significantly higher than of the leaf, supporting the significance of Mori cortex despite its smaller economical role.
- Examining the β-sitosterol content of *Morus* leaf during maturation we have concluded that the β-sitosterol content is highest in spring and decreases during the maturation process.

4. LIST OF PUBLICATIONS

Articles published in journals:

Publications related to the thesis

- Böszörményi A**,** Héthelyi É**,** Farkas Á, Horváth Gy, Papp N, Lemberkovics É, Szőke É. (2009) Chemical and Genetic Relationships among Sage (Salvia officinalis L.) Cultivars and Judean Sage (Salvia judaica Boiss.). J Agric Food Chem, 57:4663-4667.
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