**Volatile Substances Determination in Magnolia x Pruhoniciana Extracts.**

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## VOLATILE SUBSTANCES DETERMINATION IN *MAGNOLIA × PRUHONICIANA* EXTRACTS

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### Abstract

*Magnolia × pruhoniciana* is a unique hybrid originated by the crossing of Japanese *M. obovata* and American *M. tripetala*. It has been cultivated and grown since the 1950s in the Průhonice park, part of the Institute of Botany of the CAS. However, biologically active substances contained in intensely fragrant blossoms have not been sufficiently described, yet. From that reason the research was focused on extraction of the volatile substances from fresh blossoms using various solvents. Samples of magnolia blossom extracts revealed variously rich profiles of volatile substances, depending on the extraction agent used. To obtain information on volatile substances the analytical method GC/HRMS was employed. Magnolia extracts contained a large number of valuable substances such as alkaloids, coumarins, flavonoids, lignans and neolignans, phenyl-propanoids and terpenes have been identified. Obtained extracts possessed a typical magnolia fragrance, which confirmed that the obtained products could be potentially used for cosmetic applications.

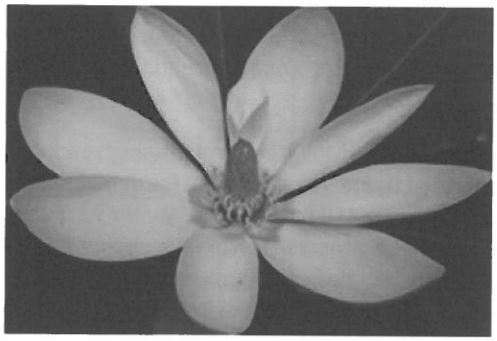

Figure 1. Flower *of Magnolia × pruhoniciana*

### Introduction

Magnolia, is a genre popular as a decorative tree [1]. Magnolia flowers often smell of fruit and are used for their effects against respiratory problems, bacterial infections, viruses and high blood pressure, leukemia, and malignant tumours. Laboratory experiments have shown that magnolia active substances inhibit tumour growth in mice in half of all cases. Intended experimental tests to investigate the possibility of some bio-refining procedures for the processing of magnolia magnolias and the isolation of the valuable substances found in magnolias such as alkaloids, coumarins, flavonoids, lignans and neolignans, phenyl-propanoids and terpenes. Magnolol (2 to 11%) and honokiol (0.3 to 4.6%) are found in the magnolia bark of the neo-ligand.

Honokiol and magnolol (Fig. 2) were initially described as components of the genus Magnolia, which are components of Chinese (Kampo) herbs, including houpo and saiboku-tu(o) [1]. In the 1990s honokiol and magnolol were found to have activity as free radical and lipid peroxidation inhibitors. Thereafter, honokiol, magnolol, and a methanol extract of Magnolia were shown to exhibit anti-oxidative, anti-inflammatory, anti-tumour, anti-diabetic complications, anti-microbial, anti-neurodegeneration, anti-depressant, pain control, hormone, gastrointestinal, and uterus modula[illegible] [illegible] protective properties [2-4].

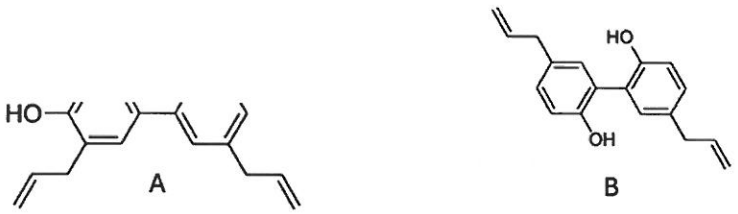


Figure 2. The chemical structures of (A) honokiol and (B) magnolol

## Experiments

Several possibilities of isolation of valuable substances from *Magnolia* × *pruhoniciana* flowers from the Botanical Institute of AS CR, v.v.i. were provided. All procedures are summarized in Tab.I.

Table I
Procedures used for isolation of valuable substances

| Flower processing procedure | Simplified describe preparation process |
|---|---|
| pressing on juicers | pressing 785.0 g of frozen flowers on a juicer, 261,3 g of juice, 474,3 g of matolin |
| steam distillation | 515 g of fresh flowers, 900 ml of water for 50 minutes (2x) |
| hexane - stirred reactor | 40.5 g of dried flowers + 750 ml of hexane in a stirred reactor |
| hexane – Soxhlet's reactor | 40.5 g of dried flowers + 750 ml of hexane in the Soxhlet's extractor |
| supercritical extraction CO2 (SFE) | 17.23 g of dried flowers are extracted with 100 L of supercritical $CO_2$ |

### Magnolia flowers pressing

Frozen magnolia flowers were processed by pressing on juicers. Two fractions were obtained from the flowers - juice and solid phase matolin and were analysed for volatile components. The experiment was performed on an Angel Juicer 7500 stainless steel juicer press, which cuts the raw material and doubles through a sieve with holes of 1 mm diameter. The material for pressing was the magnolia flowers cultivated by Institute of Botany of the Czech Academy of Sciences, Průhonice, which were frozen before transport.

### Steam distillation

Water vapour distillation: Volatile high molecular weight substances from biological materials can be gently separated by steam distillation. Fresh Magnolia flowers weighing 258 g were extracted with water vapour for 50 minutes using 450 mL of water. Water vapour distillation was carried out in two batches, about 500 g of frozen flowers were processed, from which 2x450 ml of an aqueous product with the smell of magnolia flowers was obtained. Samples of 450 mL were passed for analysis, respectively. sensory assessment from each batch. 450 mL given for analysis at the Institute of Chemical Technology and the ASCR. The total weight of the two liquid products was 1033.2 g.

### Extraction of dried magnolia flowers

Supercritical extraction

Supercritical extraction was also performed from dried magnolia flowers, and 17.23 g of dried magnolia was extracted with 100 L of $CO_2$ (0.5 L / min) at 25 MPa and 40° C. This procedure yielded 0.4015 g of the extract.

Hexane extraction

Extraction of dried magnolia was carried out with n-hexane in a stirred reactor under argon at 65° C, filtration was carried out the following day and the perfume product was obtained. The extracted flowers were dried freely. The second extraction was performed in Soxhlet extractor under a protective argon atmosphere, the next day was separated by filtration product whose smell was more intense than in the first case. The extracted flowers were dried freely.

### Preparation of extracts from fresh magnolia flowers

To avoid unwanted loss of fragrance components, the flowers were extracted fresh. The extraction of the cut flowers by leaching was carried out in two stages, while only the extracts from the first stage were used for the preparation of cosmetic scents Ethanol (70%wt. water solution according to the Czech Pharmacopoeia) and glycerol (p.a.) were used as extracting agents. Both extractions were run in parallel. The ratio of fresh flowers / extraction agent was 3g/4mL in both cases. Simultaneously, one-stage magnolia extraction with petroleum ether was also performed. Extraction by leaching took place at room temperature for 30 days. For further processing filtered extracts were used, which formed 2 phases - organic and aqueous. The phases were separated.

## Results and discussion

Comparison of sample extracts based on their fingerprints (obtained by UPLC-HRMS by separating the analyte molecules in the C18 reverse stationary phase chromatographic column and their subsequent electrospray

ionization in positive and negative mode - ESI + and ESI-) is summarised in the Table II. Obtained results give qualitative information about the richness of the analysed extracts. The Table II contains the comparison of samples from the point of view of detected compounds number.

Table II
Number of detected compounds

| Flower processing procedure | Number of detected compounds ESI+ | Number of detected compounds ESI- |
|---|---|---|
| pressing on juicers - juice | 2194 | 1015 |
| pressing on juicers - matolin | 3561 | 1154 |
| steam distillation | 392 | 18 |
| hexane - stirred reactor | 2795 | 738 |
| hexane – Soxhlet´s reactor | 2638 | 919 |
| supercritical extraction $CO_2$ (SFE) | 3413 | 984 |

Following Fig. 3 represents the only part of the spectrum detected compounds with the highest content in samples of above mentioned procedures for processing magnolia flowers.

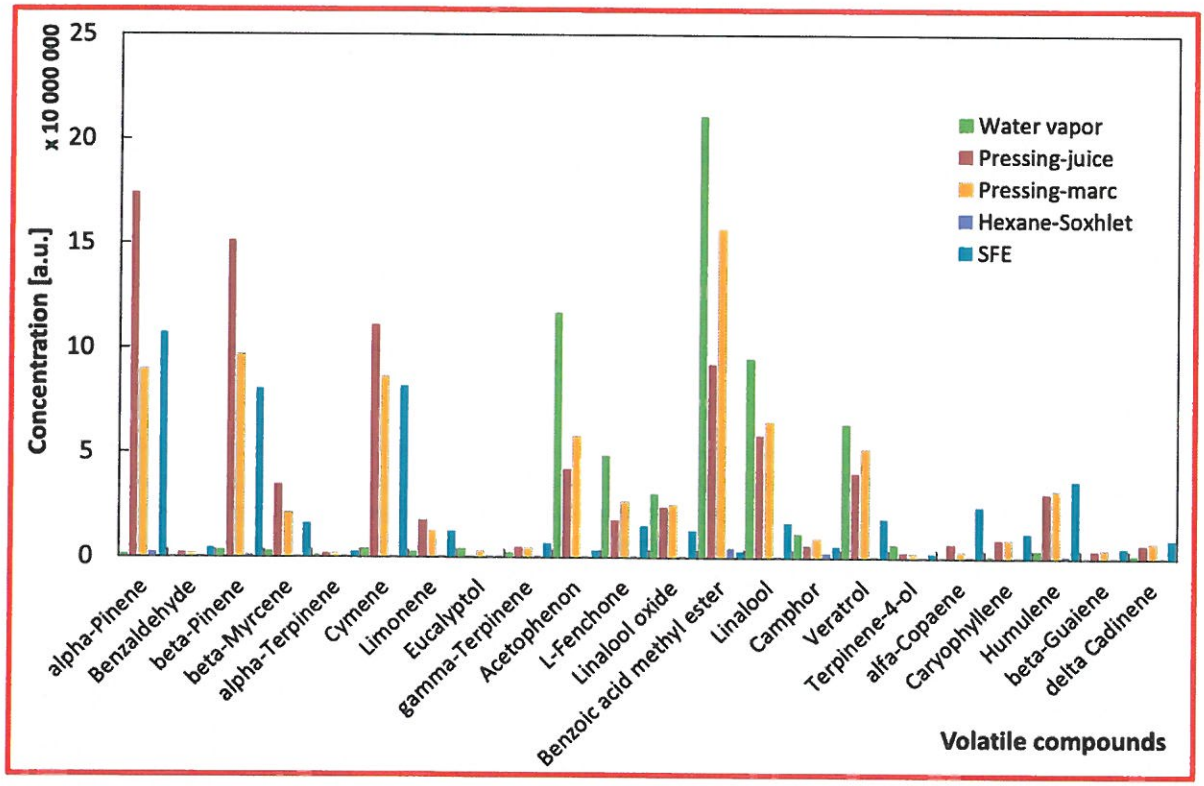


Figure 3. Effect of bioreffining methods on main components in extracts

The main interest was focused to screen biologically active substances that were previously detected in *Magnolia obovata* and *Magnolia tripetala* and consequently to select the most advantageous bioremediation procedure based on either mechanical operation (pressing of the juice and processing of the corresponding matolin) or extraction separation. solvent, extractor arrangement and time of operation. The magnolol and honokiol were detected in samples of pressing, steam distillation and hexane extraction. Most magnolol and honokiol were observed in the matolin. Lower but significant amounts of both compounds were found in the case the stirred

extractor and Soxhlet's extractor application. A negligible amount of those components was determined in the other samples.

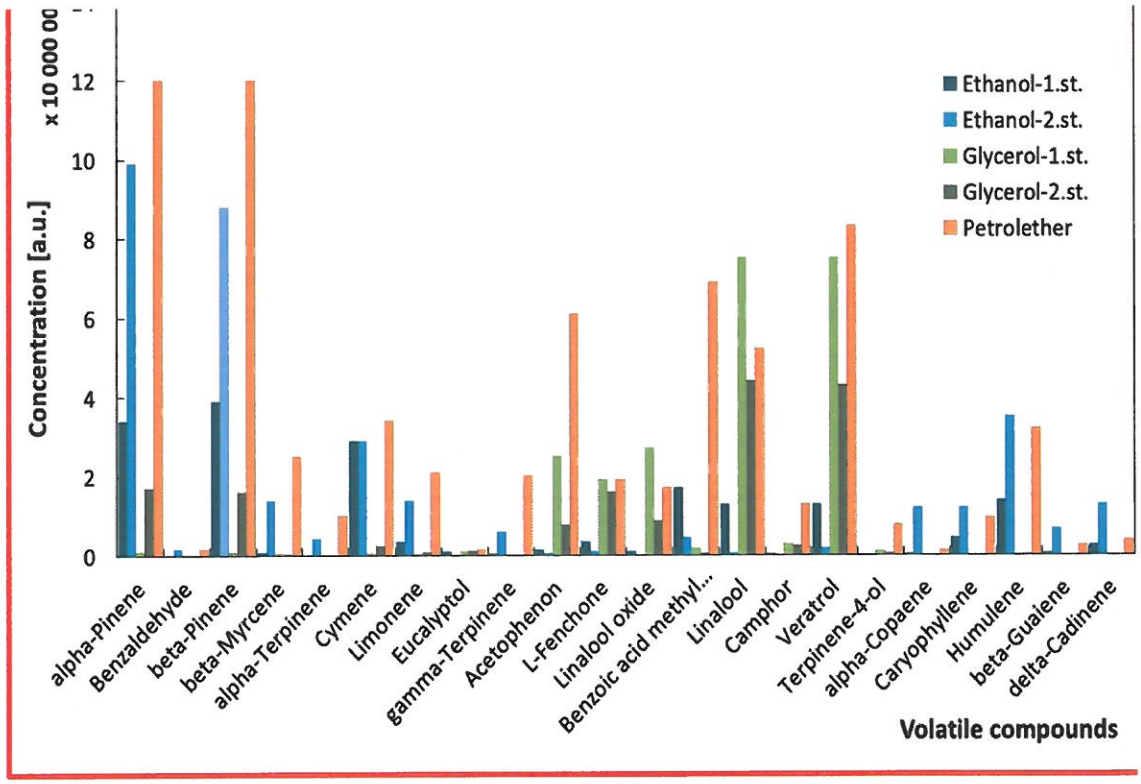


Figure 4. Profiles of main components in extract using different solvents and number of extraction stages

**Cosmetic products**

To avoid unwanted loss of fragrance components, the catted flowers were extracted in fresh form and to increase the efficiency two extraction stages were applied. For the preparation of cosmetic scents only the extracts from the first stage were used. Fig.4 presents concentration main volatile components in extracts using of ethanol, glycerol and petroleum ether. For the identification of volatile substances with a different representation, a solid-phase micro-extraction technique in headspace variant (HS-SPME) was used to trap volatile matter in the space above the sample. Samples of extracts of magnolia blossoms revealed variously rich profiles of volatile substances, depending on the extraction agent used. The reason is undoubtedly the presence of water in fresh flowers extracted in the first stage of the process. The richest profile was obtained by analysing a sample called "petroleum ether phase". Ethanol, glycerol and petroleum extracts were used to make face creams and cologne water with a *magnolia x pruhoniciana* fragrance.

Figure 5. Products for cosmetics use

## Conclusion

When pressing flowers biomass, most of the target substances remain in the waste matolin. There is obviously necessary to respect economy efficiency of the process. A small efficiency has been observed in case of the water vapour as well as extraction using supercritical extraction by carbon dioxide.

On the contrary, some prospects have both extractions with hexane, with the stirred extractor. In this case the higher concentrations of magnolol and honokiol in extracts were obtained. The reason consists in the more intense mass transfer between the solid and liquid phases. Magnolol and honokiol are promising for farmacology applications because of their antioxidant, antidepressant, anti-inflammatory, antiviral and antimicrobial effects. Fragrant essences obtained by extracting fresh flowers have been tested as face creams and fragrant ingredients that retain the pleasant smell of magnolia flowers and they could be useful for other various cosmetic products.

## Acknowledgment

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