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INFLUENCE OF PARTICLE SIZE ON UV-VIS AND NIR SPECTRA OF AQUEOUS EXTRACTS OF PLANTS FROM *LAMIACEAE* FAMILY

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Abstract

The *Lamiaceae* family plants are recognized as rich sources of phytochemicals with important antioxidant traits. Furthermore, the epidemiological studies have shown that consummation of herbal food, rich in antioxidants, has favorable impact on human health.

The aim of this study was to determine the influence of particle size on the recorded ultraviolet-visible spectroscopy (UV-VIS) and near-infrared (NIR) spectra of thyme (*Thymus serpyllum* L.), sage (*Salvia officinalis*), mint (*Mentha piperita* L.), and lemon balm (*Melissa officinalis*) water extracts. Water extracts were prepared with seven different particle sizes of the dried plants (< 100 μm, 100 μm, 250 μm, 355 μm, 500 μm, 800 μm and 1000 μm) in an oil/water bath at T = 80 ^{0}C for t = 90 min. and rpm = 500 min^{-1}. Continuous UV-VIS and NIR spectra of each extract were recorded. To determine the differences and the similarities between the extracts, the recorded UV-VIS and NIR spectral data was analyzed by the Principal Component Analysis (PCA) and Partial Least Squares (PLS) models using the Unscrambler X software, version 10.5 (CAMO Software, Norway).

PCA analysis revealed good separation of the extracts based on their particle sizes. In order to determine whether spectral data and particle size show any kind of correlation, PLS modelling was conducted. The obtained PLS models revealed that a strong linear correlation exists between particle size and absorbance data of both, UV-VIS and NIR spectra for all analyzed plant extracts. The determination coefficients for model validation were as follows: R2 = 0.511 for thyme; R2 = 0.939 for sage; R2 = 0.830 for mint and R2 = 0.828 for lemon balm.

These results confirm that the particle size influences the extraction process and the recorded spectral data of the extracts. The results also confirm the suitability of PCA and PLS analysis for correlation determination among a large data set such as spectral data.

***Key words**: Medicinal plants, Particle size, UV-VIS spectroscopy, NIR spectroscopy.*

1. Introduction

In recent years, medicinal plants have been demonstrated as valuable resources of naturally occuring bioactive compounds and many of them have shown important antioxidant properties [1]. Epidemiological studies have shown that consummation of plants, rich in antioxidants, has favourable effects on human health [2]. Among them, *Lamiaceae* family represent a rich source of polyphenolic compounds that are used in traditional and modern medicine, food industry, cosmetics and pharmaceutical industry. Various species of the genera are widespread throughout the world and are often used for the treatment of wounds, gastritis, infections, dermatitis, bronchitis, and inflammation [3].

Numerous analytical methods have been developed for the analysis of chemical composition of medicinal plants extracts and quality control [4]. All these methods are precise but expensive, time-consuming, require chemicals and are not environmentally-friendly. In order to ensure product development and quality control, the use of Near Infrared Spectroscopy (NIRS) has emerged over the past 20 - 30 years. NIRS has become one of the most frequently used method of analysis, providing simultaneous, rapid and non-destructive quantification of the major components in many agricultural products and plant materials [5 - 9].

Since NIR absorption spectra are often complex and possess broad overlapping NIR absorption bands, special mathematical procedures are required for the analysis of obtained data. NIR identification and qualification can be performed by comparing a sample spectrum to reference spectra of known materials while quantification can be done by using mathematical models and multivariate analysis (chemometrics) [10]. The combination of vibrational spectroscopy and chemometrics provides calibration models for specific complex-matrix analyses and it is suitable to handle spectral interferences and spectral noise on vibrational spectra, providing good data acquisition and data-processing methods [11, 12].

The aim of this study was to determine the influence of particle size on the recorded UV-VIS and NIR spectra of four medicinal plants: thyme (*Thymus serpyllum* L.), sage (*Salvia officinalis*), mint (*Mentha piperita* L.) and lemon balm (*Melissa officinalis*) water extracts.

2. Materials and Methods

2.1 Plant materials

Thyme (*Thymus serpyllum* L.), sage (*Salvia officinalis*), mint (*Mentha piperita* L.) and lemon balm (*Melissa officinalis*) were purchased from specialized herbal store (Suban d.o.o., Zagreb, Croatia). Plant materials were collected during the flowering season of 2015 in the north-western part of Croatia, dried naturally and stored at ambient conditions before further use.

2.2 Milling

Dried plant material was milled using IKA Tube mill control (IKA-Werke, Staufen, Germany) and Gorenje TGO mill (Gorenje, Velenje, Slovenia). Milling conditions were as follows: 15000 rpm with adjusted milling time (t = 10 - 40 s.) in order to obtain different particle size fractions. After milling, samples were kept in a desiccator until further used.

2.3 Sieving

Milled plant material was subjected to sieving in order to separate the particle size fractions. The following standardized DIN sieves (Fritsch, Idar-Oberstein, Germany) were used: 100, 250, 355, 500, 800 and 1000 µm pore diameter. Obtained particle size fractions were then stored at T = 20 ^{0}C and RH = 40% before use.

2.4 Extraction procedure

Thyme, sage, mint and lemon balm water extracts were prepared by aqueous extraction. An amount of m = 2 g of dry plant material was placed in a V = 200 mL glass with V = 100 mL of deionised water, covered with aluminium foil, and heated to a T = 80 ºC ± 0.5 ºC using Ika HBR4 digital oil-bath (IKA-Werk GmbH & Co.KG, Staufen, Germany). Experiments were performed with seven different particle sizes of the dried plants (< 100 µm, 100 µm, 250 µm, 355 µm, 500 µm, 800 µm and 1000 µm), at the magnetic stirrer rotational speed of 500 rpm during t = 90 min. Samples were immediately cooled in the water-ice mixture, filtered through a 100% cellulose paper filter (LLG Labware, Meckenheim, Germany) with d = 5 - 13 µm pore size and stored at T = 4 ºC until analysed.

2.5. NIR spectroscopy

Measurements of the spectra were performed using NIR spectrophotometer NIR128L-1.7 (Control Development, South Bend, Indiana, USA) with installed Control Development software Spec32 using a halogen light source (HL-2000) with a spectral resolution of 6.25 cm^{-1}. The NIR spectra (904 - 1699 nm) of plant water extracts for different particle sizes were collected with the setup for NIRS studies previously described by Valinger *et al.*, [13]. Three consecutive runs for every particle size of plant water extracts were initially recorded across the entire spectral range and the average spectrum was used for analysis. No mechanical or chemical treatment of the samples was needed prior to NIRS measurements.

2.6 UV-VIS spectroscopy

Plant water extracts for different particle sizes were scanned by a UV-VIS spectrophotometer (Biochrom Libra S11, Cambridge, England) with Acquire Lite software, in the range 325 - 900 nm. UV-VIS spectra were recorded three times for every particle size of plant water extracts and the average spectrum was used for analysis.

2.7 Data analysis and chemometric models

Principal component analysis (PCA) is a multivariate technique that analyzes a data table representing observations described by several dependent variables, which are inter-correlated. PCA goal is to extract the important information from the data table and to express this information as a set of new orthogonal variables called principal components [14]. PCA also represents the pattern of similarity of the observations and the variables by displaying them as points in maps [15 - 18].

Partial least squares (PLS) regression is a method based on the multiple regression and the principal component analysis for use in chemometrics [19]. PLS was used for prediction of a set of dependent variables from a set of independent variables (predictors). This prediction is achieved by extracting from the predictors a set of orthogonal factors called latent variables which have the best predictive power [9]. PLS regression is particularly useful while working with multicolinear data, a

large set of predictive variables can be included and several response variables can be modelled simultaneously [19, 20].

The recorded UV-VIS and NIR spectral data were analyzed by the PCA and PLS models using the Unscrambler X software, version 10.5 (CAMO Software, Oslo, Norway).

3. Results and Disscusion

3.1 UV-VIS and NIR spectra

Using the UV-VIS spectrophotometer and the NIR instrument, three recordings were made for every fraction of particles (< 100 µm, 100 µm, 250 µm, 355 µm, 500 µm, 800 µm, and 1000 µm) for each plant water extract (thyme, sage, mint and lemon balm), resulting in 84 recordings of pre-processed raw spectra. Examples of combined UV-VIS and NIR raw spectra for thyme, sage, mint and lemon balm water extracts are presented in Figures 1 - 4.

According to the data presented in Figure 1, the greatest differences were observed in the UV-VIS spectra range between 400 nm and 600 nm while for the NIR range (between 1400 nm and 1699 nm), spectral shift of the spectra recorded for thyme water extracts was observed.

Combined UV-VIS and NIR raw spectra for different particle sizes of sage water extracts are presented in Figure 2.

The greatest differences can be observed in the UV-VIS spectra range from 400 nm to 500 nm. Spectral shift of the spectra recorded for sage water extracts was observed for the NIR range between 1400 and 1699 nm.

Resuts from combined UV-VIS and NIR spectra of mint water extracts are presented in Figure 3.

Spectra analysis obtained for different particle sizes of mint water extracts showed the lowest differences between individual spectra of mint extracts, compared to the spectra of other plant extracts. The greatest difference can be observed in the UV-VIS spectra range between 550 nm and 600 nm (Figure 3). Spectra obtained in the NIR range did not show visible differences or spectral shifts.

Results for combined UV-VIS and NIR spectra of lemon balm water extracts are presented in Figure 4.

Regarding lemon balm water extracts (Figure 4), the greatest differences can be noticed in the UV-VIS range from 400 nm to 600 nm. As in the case of mint, spectra obtained in the NIR range did not show visible differences or spectral shifts.

3.2. Principal component analysis (PCA)

Since large amount of data has been obtained from the recorded UV-VIS and NIR spectra, 2D Principle Component Analysis (PCA) was applied in order to investigate similarities and differences of four medicinal plant extracts based on their particle sizes. PCA analysis is one of the most often used chemometric techniques for extracting useful information from recorded spectra [21].

PCA analysis of thyme water extracts (Figure 5a) shows differences between samples with smaller (< 100 µm and 100 µm) and larger (250, 355, 500 and 1000 µm) particle sizes. It can be observed that samples with the particle sizes of 250, 355, 500 and 1000 µm are clustered around the same point, indicating a great similarity of the examined factors.

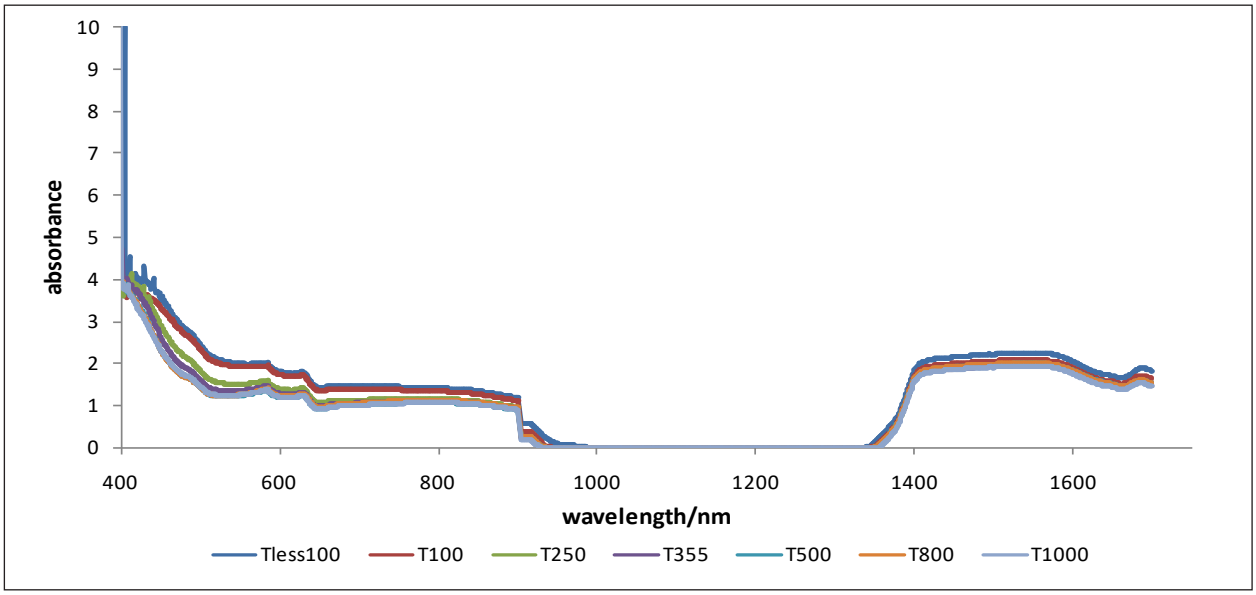

Figure 1. Samples of combined UV-VIS and NIR spectra of thyme (T) water extracts corresponding to the raw data for the particle sizes < 100 µm (Tless100), 100 µm (T100), 250 µm (T250), 355 µm (T355), 500 µm (T500), 800 µm (T800) and 1000 µm (T1000)

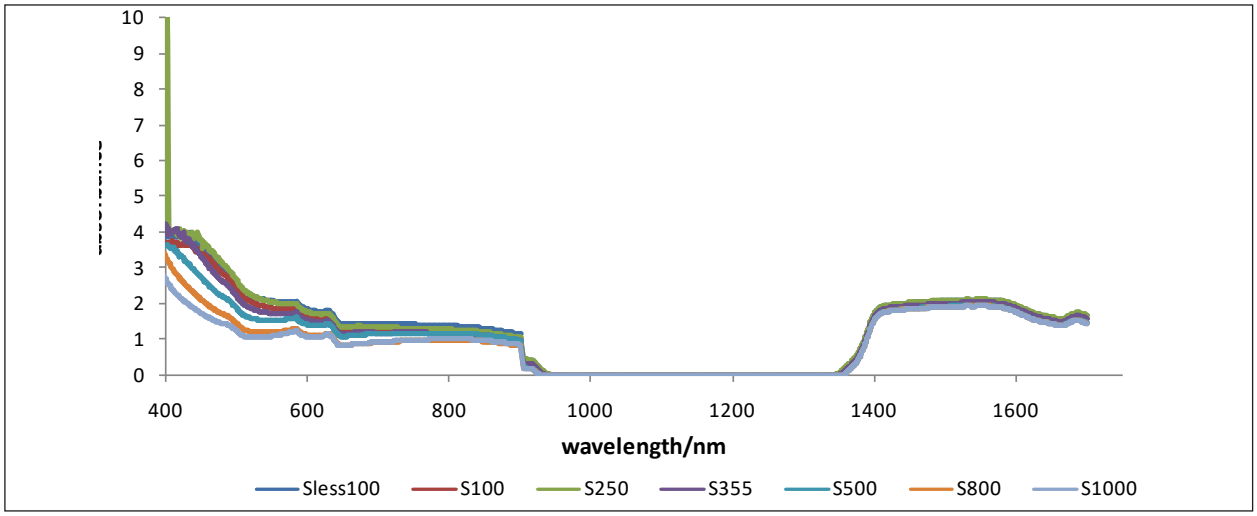

Figure 2. Samples of combined UV-VIS and NIR spectra of sage (S) water extracts corresponding to the raw data for the particle sizes < 100 µm (Sless100), 100 µm (S100), 250 µm (S250), 355 µm (S355), 500 µm (S500), 800 µm (S800) and 1000 µm (S1000)

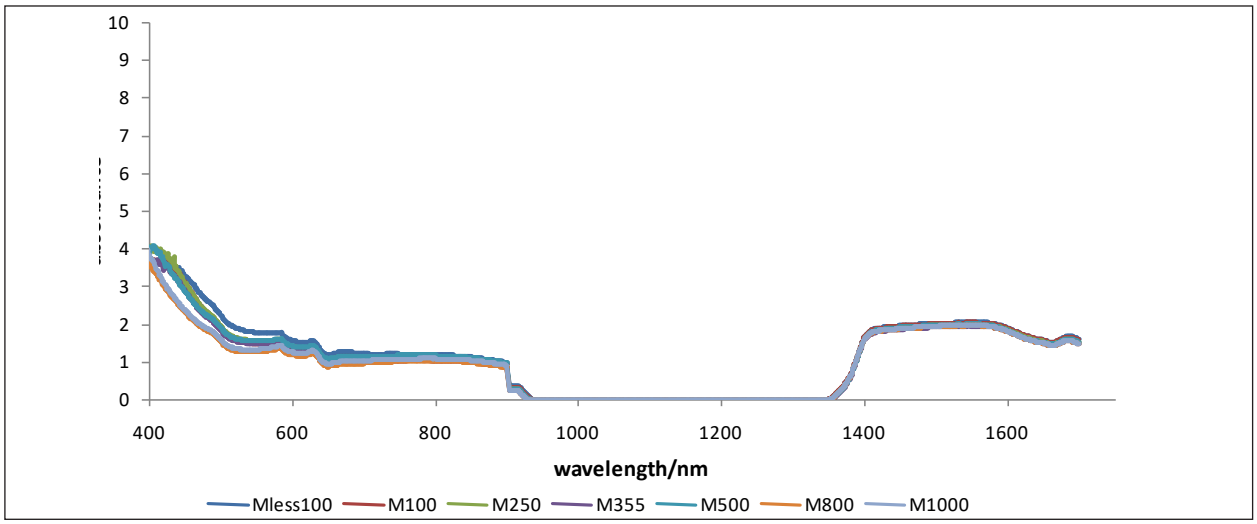

Figure 3. Samples of combined UV-VIS and NIR spectra of mint (M) water extracts corresponding to the raw data for the particle sizes < 100 µm (Mless100), 100 µm (M100), 250 µm (M250), 355 µm (M355), 500 µm (M500), 800 µm (M800) and 1000 µm (M1000)

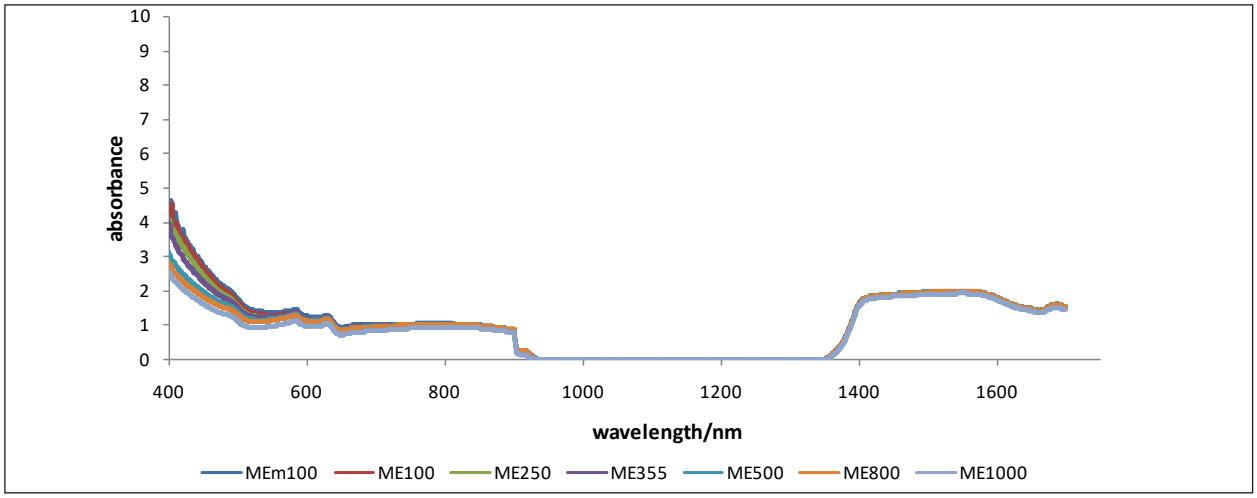

Figure 4. Samples of combined UV-VIS and NIR spectra of lemon balm (LB) water extracts corresponding to the raw data for the particle sizes < 100 µm (LBless100), 100 µm (LB100), 250 µm (LB250), 355 µm (LB355), 500 µm (LB500), 800 µm (LB800) and 1000 µm (LB1000)

According to PCA analysis of sage water extracts (Figure 5b), samples with the particle sizes < 100 μm, 100 μm, 355 μm and 500 μm are clustered in the lower quadrants while samples with the partice sizes 250 μm, 800 μm and 1000 μm are clustered in the upper quadrants. Since samples with the particle sizes 800 μm and 1000 μm are located close to each other (the second quadrant) it can be concluded that there is a great similarity between them. Significant difference between the particle sizes of 1000 μm and less then 100 μm can be noticed because they are diagonally distributed in the first and the third quadrant.

For mint water extracts (Figure 5c), PCA analysis clustered samples with smaller particle sizes (< 100 μm, 100 μm, 250 μm and 355 μm) in the left quadrants while samples with larger particle sizes (500 μm and 1000 μm) were clustered in the right quadrants, with the exception of the particle size fraction of 800 μm (probably due to measurement error or other malfunction). Samples with the particle sizes of 500 μm and 1000 μm are distant from other samples, indicating that they differ from other samples.

Regarding lemon balm water extracts (Figure 5d), samples with the particle sizes < 100 μm, 100 μm, 250 μm and 355 μm are clustered in the left quadrants while samples with the particle sizes 500 μm, 800 μm and 1000 μm are clustered in the right quadrants (the first and the second quadrant), indicating that they differ from samples with smaller particle sizes.

According to the obtained results (Figures 5b, c, d) it can be concluded that Principal Component Analysis showed good differentiation between samples with smaller particle sizes (< 100 μm, 100 μm, 250 μm and 355 μm) and samples with larger particle sizes (500 μm, 800 μm and 1000 μm) for sage, mint and lemon balm while this was not a case for samples of thyme water extracts (Figure 5a). In the work of Valinger *et al.*, [22], PCA analysis revealed very good differentiation between five dried medicinal plants for the particle sizes <100 μm, which was not the case for the other particle sizes (100 - 280 μm and 280 - 450 μm). Similar results were obtain in the work of Gajdoš Kljusurić *et al.*, [18] where PCA analysis was used to confirm that NIRS can distinguish different medicinal plants with the same fraction size.

Distribution of plant materials can be illustrated in three-dimensional plot of PC1 versus PC2 and PC3 (Figure 6). Samples of sage (yellow circle) lemon balm (red triangle) and thyme water extracts (blue diamond) are clearly separated with introduction of the

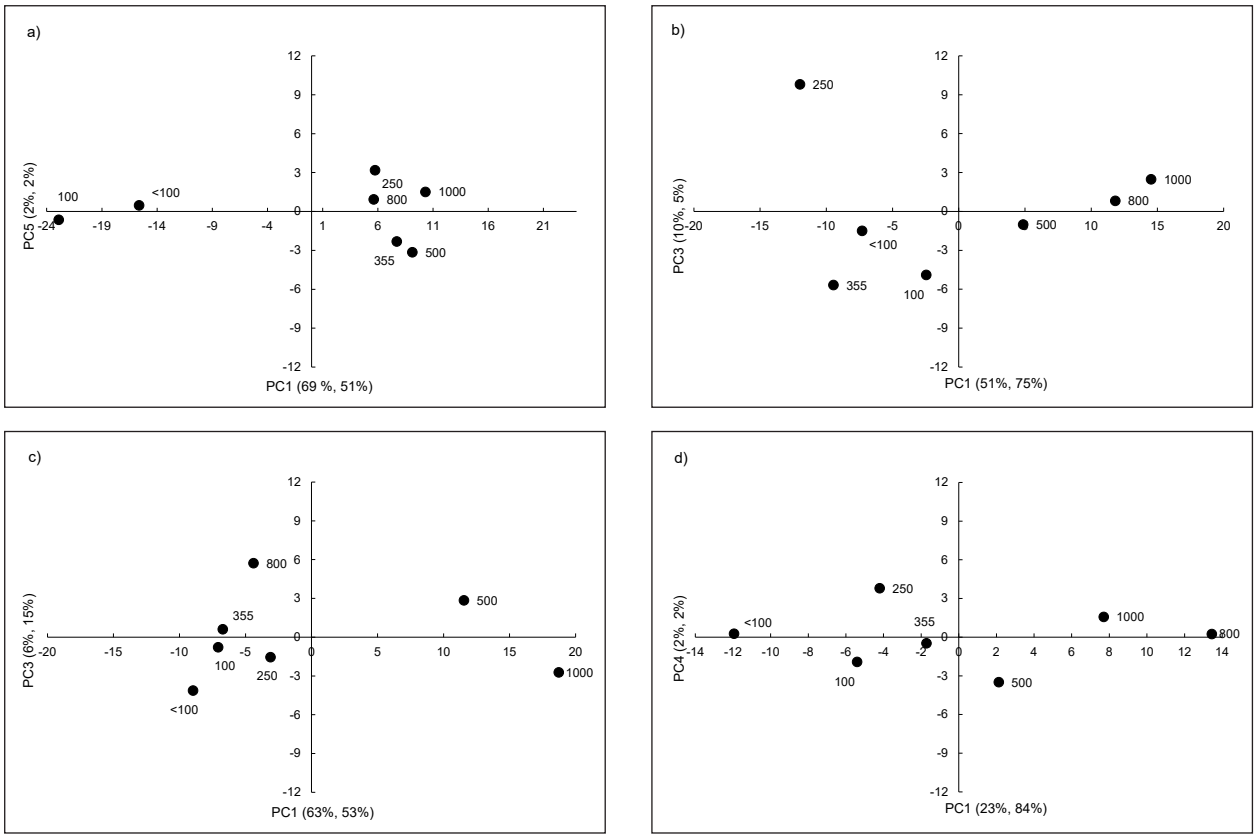

Figure 5. Principal component analysis plots for a) thyme, b) sage, c) mint and d) lemon balm. Values in the brackets represent the factor values for calibration and validation. Factors presented in the images were chosen based on the efficacy of separation of the extracts by particle size of the plant material used to perform the extraction.

third factor in 3D PCA. For mint water extracts (blue asterisk), certain trend cannot be determined due to considerable scattering between experimental data. This justified the usefulness of PCA analysis in finding differences between large data sets such as the results of spectral analysis [18].

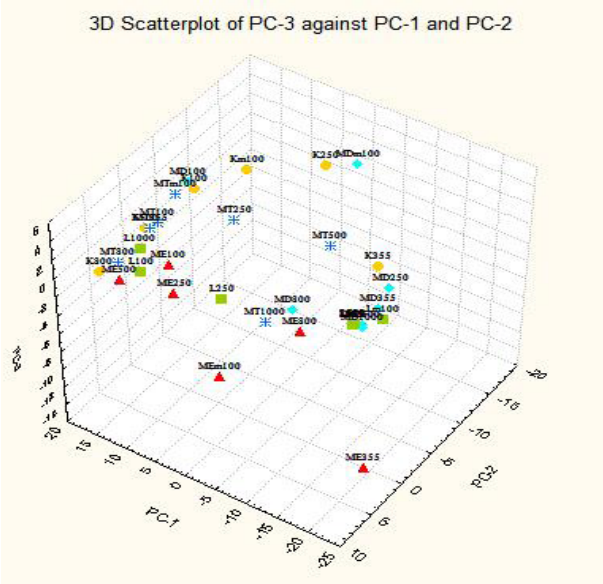

Figure 6. Principal Component Analysis of raw spectra presented by the first three factors (PC1, PC2, PC3) for particle size fractions (< 100 µm, 100 µm, 250 µm, 355 µm, 500 µm, 800 µm and 1000 µm) for thyme (blue diamond), sage (yellow circle), mint (blue asterisk) and lemon balm (red triangle) water extracts

3.3 Partial least squares (PLS) regression

In order to determine whether spectral data and particle sizes show any kind of correlation, Partial Least Squares modeling was conducted. Figures 7 - 10 present PLS regression models where the independent variables were combined UV-VIS and NIR spectra while dependent variables were different particle sizes of analyzed plant water extracts. Goodness of fit of PLS regression was evaluated based on the calculated determination coefficient (R^2) for calibration and model validation and root mean squared error values (RMSE) (Table 1).

Although application of PLS regression showed linear correlation between combined UV-VIS and NIR spectra and particle sizes of thyme water extracts (Figure 7a), calculated R^2 value for model validation was low (R^2 = 0.511) compared to R^2 value for model calibration (R^2 = 0.999) (Table 1). PLS model can be used for calibration but cannot be used for future prediction of particle sizes of thyme samples based on spectral data.

According to calculated R^2 values for calibration (R^2 = 0.999) and validation (R^2 = 0.939) (Table 1), PLS regression gave the most suitable description of experimental data for sage water extracts. Figure 7b shows strong linear correlation between dependent and predictor variables which means that PLS model could be used for future prediction of particle sizes of sage samples based on spectral data.

According to the obtained results (Figure 7c), it can be seen that linear correlation exists between combined UV-VIS and NIR spectra and particle sizes for mint extracts. Based on calculated R^2 value (R^2 = 0.946) for model prediction and R^2 value (R^2 = 0.830) for model validation (Table 1), it can be concluded that particle sizes of mint samples could be reasonably predicted based on input data.

As in case of mint samples, good prediction of particle sizes of lemon balm water extracts can be obtained based on input (spectral) data. Determination coefficients of model calibration and validation were R^2 = 0.995 and R^2 = 0.828, respectively (Table 1) while Figure 7d presents linear correlation between dependent and predicted variables.

Root mean squared error values (RMSE) obtained for model calibration were lower, compared to the values obtained for model validation (Table 1). The lowest RMSE value was obtained for thyme (7.6637) while the highest value has been noticed for mint (76.5230). RMSE values for model validation were in range from 94.8318 (sage) to 268.3261 (thyme). Determination coefficient (R^2) values were higher for model calibration for all four analysed plants in comparison to the validation R^2 values. The R^2 values exhibited strong linear correlation between particle size and the combined

Table 1. PLS model values for aqueous plant extracts made with different particle sizes of the plant material. RMSE represent the root mean squared error and the R^2 the coefficient of determination of the calibration and validation models

Plant extract		Slope	Offset	RMSE	R^2
Thyme	Calibration Validation	0.9994 0.4144	0.2368 171.1697	7.6637 268.3261	0.9995 0.5113
Sage	Calibration Validation	0.9989 0.8890	0.4587 50.7512	10.6678 94.8318	0.9989 0.9390
Mint	Calibration Validation	0.9459 0.6084	23.6087 146.7470	76.5230 158.1254	0.9459 0.8303
Lemon balm	Calibration Validation	0.9952 0.6987	2.0760 107.0250	22.6886 159.1728	0.9952 0.8280

UV-VIS and NIR spectral data, with an exception of the R^2 value for the validation of thyme extracts (R^2 = 0.511). Possible explanation for the dissipation of the spectral data, as well as for the lower R^2 values could be the separation of different parts of plants (e.g. petals, stems, leaves etc.) during the sieving process, which could have resulted in different compounds extracted during the extraction process and the differences in spectral data. Based on the calculated R^2 values it can be concluded that PLS regression is an acceptable method for UV-VIS and NIR spectral data analysis and modelling.

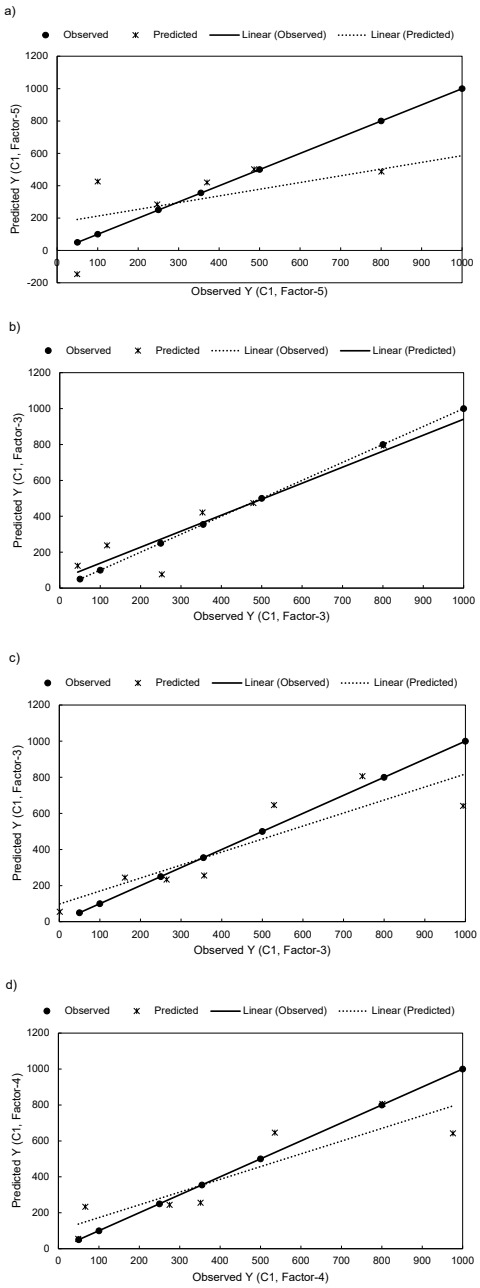

Figure 7. Observed/predicted plots of PLS model predictions of particle size based on UV-VIS and NIR spectra for a) thyme, b) sage, c) mint and d) lemon balm

4. Conclusions

- Application of UV-VIS and NIR spectroscopy in combination with PCA and PLS regression showed a good potential in differentiation of investigated medicinal plants based on their particle size fractions.

- According to the obtained results it can be concluded that UV-VIS and NIR spectroscopy, in combination with chemometric tools, can be used for monitoring physical properties of medicinal plant water extracts in terms of quality control of the final product.

5. References

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