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Comparative Analysis of Silybum Marianum Fruit Extracts and Thyme Oil Samples Using NMR-based Multivariate Statistical Methods

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1. Introduction

The fruits of the medicinal plant milk thistle (*Silybum marianum* (L.) Gaertn. Asteraceae) have long been used to treat certain hepatic conditions like cirrhosis or protecting liver tissue from drug side effects e.g. in chemotherapy. This hepatoprotective and regenerating effect is linked to the antioxidant silymarin, a complex mixture of flavonolignans mainly concentrated in the pericarp of the fruit. The essential oil extracted from various *Thymus* species is known for its distinct antifungal and antibacterial properties, which are mediated by the various monoterpene components found in the oil, especially thymol and p-cymene.

Multivariate statistical methods simplify the analysis of complex sets of data by analyzing several related and interacting variables at the same time, thus negating the limitations of univariate analyses that cannot cope with the interactions of different variables. The aim of this study is to compare the nuclear magnetic resonance (NMR) spectra of milk thistle fruit extract and thyme oil samples respectively by using two statistical methods, namely principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA). Furthermore, we sought to find a relationship between the spectral features of the different milk thistle extracts and their antioxidant capacity.

2. Materials and methods

For our study whole fruit samples of *Silybum marianum* were obtained from four different sources, including one white-flowered Hungarian cultivar called 'Szibilla'. Additionally, four differently branded thyme oil samples were purchased from retail stores in Hungary.

Extraction of the fruits was carried out with methanol under reflux, then the solvent was evaporated. The dry extracts were reconstituted in DMSO-d6 and their quantitative ^{1}H NMR spectra were recorded at 600 MHz. After their uniform processing, the spectra were integrated into 0.02 ppm wide bins. For the statistical analysis,

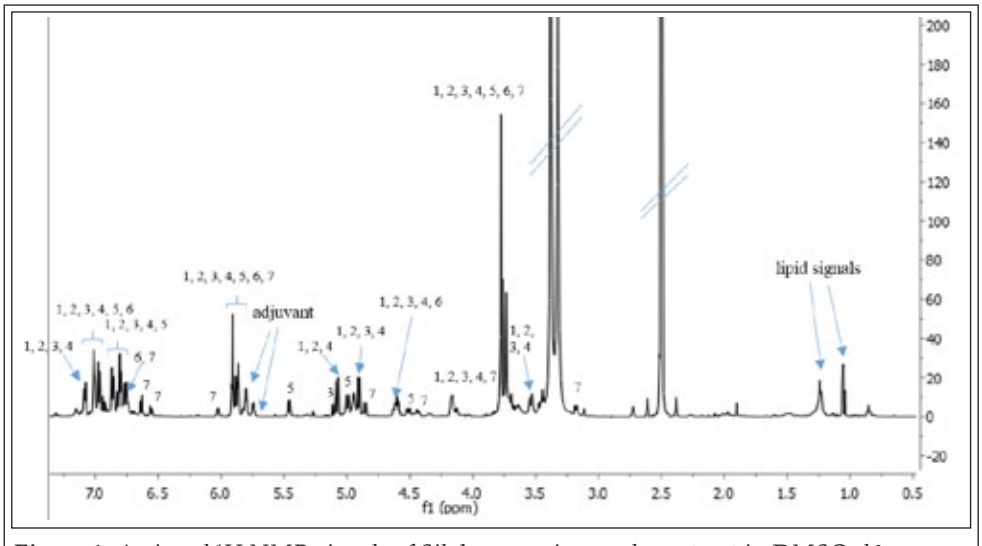

Figure 1 *Assigned ^{1}H NMR signals of Silybum marianum dry extract in DMSO-d6*
1. silybin A; 2. silybin B; 3. isosilybin A; 4. isosilybin B; 5. silychristin; 6. isosilychristin; 7. silydianin

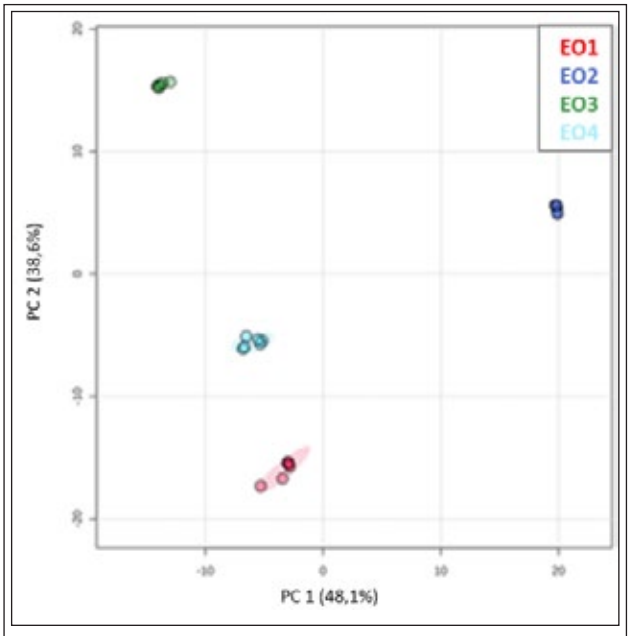

Figure 2 *PCA scores plot of the four thyme essential oil (EO) samples (sum normalization, pareto scaling)*

data matrices were built from the integral values and introduced to the online open-source metabolomical software MetaboAnalyst [1]. Fixed reaction time DPPH assay technique was used for the comparison of the antioxidant capacity of the samples [2].

As for the thyme oil samples, they were readily miscible with $CDCl_3$ at a 10 µl : 690 µl ratio that was applied for the NMR analysis with consideration to component concentrations and peak shifts. After that, the NMR and statistical analyses were similar to those of the milk thistle samples.

3. Results

The ^{1}H NMR spectra of the milk thistle samples (***Figure 1***) show mainly signals of the flavonolignan compounds, assigned according to literature data [3]. Additionally, lipid and adjuvant material signals can be seen. The PCA and PLS-DA analyses were able to characterize the differences between the samples. The use of various data preprocessing strategies had a significant influence on the outcomes of the statistics, which were taken into consideration during the interpretation of the results.

The most prominent differences emerged from the segments containing overlapping signals of antioxidant components, as well as from one specific, structurally different component, silydianin. Ultimately, the samples were classified into two distinct chemotypes. Comparing the samples' antioxidant capacity, we found that the ones that shared similar component profile showed correlation with the integral value of the area containing the overlapping signals indicating overall flavonolignan content.

After the analysis of the spectra and statistics of the thyme oil samples, it was determined that even though they belonged to the same chemotype, they differed considerably in their component profiles (***Figure 2***). The statistical outcomes once again were influenced by the choice of the data preprocessing steps. The component most frequently appearing in the statistics was linalool, though differences in thymol and p-cymene content among others could also be detected. In two of the four samples significant amounts of an unlabeled dilutant, dimethyl-phthalate were discovered.

4. Conclusions

The applied statistical methods uncovered differences in the phenoloid composition of the milk thistle extracts and the monoterpenoid component profiles of the thyme oil samples. However, as regards the milk thistle extracts, the many structurally closely related compounds of the flavonolignan complex make it difficult to evaluate the results from the NMR spectra alone.

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