

## EVALUATION OF THE LIPOPHILICITY AND STABILITY OF PHENOLIC COMPOUNDS IN HERBAL EXTRACTS

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**Background.** Phenolic compounds are secondary plant metabolites, which occur in different parts of cell, according to their lipophilicity. The objective of this study was to determine differences in the content and stability of those compounds in herbal extract fractions marked by increasing lipophilicity.

**Material and methods.** Eight herbal plants were analysed: basil, savory, lovage, lemon balm, peppermint, parsley, oregano and sage. Extracts were prepared from fresh plants harvested in late July, and they were separated into aqueous fractions and isolated with a 40% and 70% methanol solution on C18 silica gel. The total content of phenolics was determined using the Folin-Ciocalteu method directly after preparation and after three and six months of storage.

**Results.** The stability of fresh herb extracts varied subject to plant species. The lemon balm leaf extract was most abundant in phenolic compounds and showed the highest polyphenol concentrations throughout the storage period. The greatest drop in phenolic compound levels was observed in parsley extracts. Following the separation of raw extracts into three fractions, the highest phenolic compound concentrations were noted in fractions isolated with a 40% methanol solution. The most dynamic changes in polyphenol content were reported in the initial period of storage (three months) regardless of the fraction's lipophilicity. Compounds that were not degraded after this period were marked by high stability.

**Conclusions.** Phenolic compounds found in herbal plants are hydrophilic to lipophilic substances which are stable compounds regardless of their affinity for water.

**Key words:** herbs, phenolic compounds, storage

### INTRODUCTION

The relationship between diet and human health had been recognised for a long time, but the first attempts to use nutritional factors in the prevention and treatment of

diseases date back to the first half of the 20th century when essential vitamins and minerals were discovered. The data supplied by intensive research in the past 25 years as well as the observations of population groups that are less affected by lifestyle diseases show that apart from nutrients, food contains non-nutritional compounds that may effectively prevent and even treat various diseases, including serious ailments such as atherosclerosis and malignancies [Bravo 1998].

In addition to the consumption of fruit and vegetables, the recommended source of vital compounds, diet supplementation with herbs rich in antioxidants delivers a variety of health benefits. Natural products which are a rich source of bioactive substances with antioxidant properties have attracted the interest of pharmaceutical companies, food processing and cosmetic industries, as well as individual consumers [Capecka et al. 2005, Zheng and Wang 2001]. Herbs and herbal extracts are natural food preservatives, and they offer an alternative to synthetic substances used in food production, mainly meat processing. The organoleptic properties of herbs are determined by essential oils, most of which are volatile substances characterised by limited stability in herbal products [Kostrzewa 1997, Shan et al. 2005]. Herbs also contain various polyphenol compounds which are deprived of taste or aroma but deliver various health benefits. Polyphenols comprise mostly phenolic acids and flavonoids which have been shown to possess a broad spectrum of biological activity [Cosio et al. 2006, Wong and Kitts 2006].

Owing to their chemical structure, polyphenols are marked by a different degree of solubility in water. Phenolic acids containing the carboxyl group are dissociated which makes them hydrophilic [Rice-Evans 1997]. Flavonoids are apolar molecules which are mostly lipophilic. Yet owing to the variety of naturally occurring flavonoid and phenolic acid derivatives, the hydrophilic and lipophilic nature of a given compound group cannot be determined unambiguously [Harborne 1988].

Previous studies investigating the content and the chemical activity of phenolic compounds in herbal plants did not provide any information on their separation into fractions with increasing lipophilicity. In most cases, phenolic compound fractions are extracted with a 60% to 100% aqueous methanol solution and are analysed as a whole [Capecka et al. 2005, Cosio et al. 2006, Proestos et al. 2005]. In this study, phenolic compounds extracted from herbal plants with the use of a 80% ethanol solution were further divided into three sub-fractions with varied affinity for water. The objective of this study was to determine differences in the content and stability of phenolic compounds in the resulting sub-fractions.

## MATERIAL AND METHODS

The experimental material comprised the leaves of eight herb plants: basil, savory, lovage, lemon balm, peppermint, parsley, oregano and sage (Table 1). The plants were grown in experimental plots near Zamość, and they were harvested in late July. Fresh and healthy leaves were washed, dried and used in analyses within 24 h after harvesting. The dry matter content was determined with the use of the drying method by weighing 1 g chopped plant material in three replications. Ethanol extracts were prepared simultaneously by homogenizing 0.5 g chopped herbs in 25 cm<sup>3</sup> ethanol (80%). The part of obtained extract (10 cm<sup>3</sup>) was evaporated in a vacuum evaporator at a temperature of 40°C, it was weighed, and the dry residue after dissolution in 5 cm<sup>3</sup> water was placed

Table 1. Extraction yield and percent of compounds in fractions with increased lipophilicity

Plant	Ethanol extract %	Fractions, %wt					
		H <sub>2</sub> O		methanol 40%		methanol 70%	
		A <sup>a</sup>	B	A	B	A	B
Basil <i>Ocimum basilicum</i>	6.9	4.3	61.6	1.1	16.2	1.4	20.3
Savory <i>Satureja hortensis</i>	9.9	3.5	35.5	2.7	26.9	1.6	16.2
Lovage <i>Levisticum officinale</i>	10.4	5.8	55.3	1.9	18.3	1.4	13.5
Lemon balm <i>Melissa officinalis</i>	8.5	4.1	47.9	2.2	25.6	2.0	23.7
Peppermint <i>Mentha piperita</i>	9.4	3.2	33.7	2.1	22.0	1.9	20.3
Parsley <i>Petroselinum crispum</i>	18.3	11.7	64.1	2.4	12.9	2.9	15.9
Oregano <i>Origanum vulgare</i>	8.2	3.9	46.9	2.3	28.0	1.2	14.6
Sage <i>Salvia officinalis</i>	8.7	4.1	46.8	2.6	29.6	1.7	19.7

A – from plant material.

B – from ethanol extract.

on a Sep-Pack C18 column (Waters). After the application of samples, hydrophilic compounds were eluted with water (fraction I), less polar fractions were eluted with a 40% aqueous methanol solution (fraction II), and the most lipophilic fractions were eluted with a 70% methanol solution (fraction III). Every extraction was performed with 5 cm<sup>3</sup> eluent. The resulting fraction were evaporated in a rotary evaporator under reduced pressure at a temperature of 40°C. Dry residue was weighed and dissolved in a 50% methanol solution to a total volume of 2 cm<sup>3</sup>.

Extraction efficiency was calculated as the ratio of fraction mass to the dry matter of plant material (Table 1, column A) and the dry matter of ethanol extract (Table 1, column B). The total content of phenolic compounds was determined by the Folin-Ciocalteu method [Zheng and Wang 2001]. The fractions were analysed directly after preparation and after three and six months of storage in glass vials at a temperature of 5°C. The phenolic compound content was calculated in mg of chlorogenic acid based on a reference curve. All analyses were carried out in two replications. Standard deviation (SD) was calculated for each data series as an indicator of dataset scatter. The significance of differences between means was determined with the Tukey multiple range test with 5% error probability. Test results were used to identify groups of means showing significant differences and to determine LSD values.

## RESULTS AND DISCUSSION

An analysis of the efficiency of extraction with a 80% ethanol solution, calculated based on the dry matter of 10 cm<sup>3</sup> ethanol extract, showed that the extracted compounds had a 6.9% to 18.3% share of the mass of initial plant material (Table 1). The separation of ethanol extract into an aqueous fraction (fraction I) and fractions extracted with a 40% (fraction II) and 70% methanol solution (fraction III) enabled to divide hydro-

philic and lipophilic compounds into three groups with increasing lipophilicity. The highest extraction efficiency was observed in fraction I which accounted for 3.5% to 11.7% of plant material mass. The aqueous fraction contained 33.7% to 64.1% ethanol extract compounds (Table 1). Fractions II and III were characterised by a similar degree of extraction efficiency which reached 1.1% to 2.9% of plant material mass. As regards the ethanol extract, 12.9% to 29.6% efficiency was reported. The obtained results suggest that ethanol extracts from eight herbal plants contained higher levels of hydrophilic compounds in the aqueous fraction.

The efficiency of ethanol extraction noted in this study was below the values noted by Škerget et al. [2005] who reported efficiency levels of 24–34%. These differences could be due to an alternative method of preparing plant tissue extracts. The cited authors used dried and ground plant material, from which phenolic compounds were extracted with 100% methanol in an ultrasonic bath.

The total phenolic compound content was determined by the Folin-Ciocalteu method. The phenolic compound content of ethanol extracts varied subject to plant species (Fig. 1). The highest concentrations were noted in the lemon balm extract at 21.6 mg·g<sup>-1</sup> fresh weight, calculated as chlorogenic acid. Basil, lovage and parsley extracts proved to be the least abundant sources of phenolics at 4.9 mg, 5.3 mg and 4.6 mg, respectively. Similar phenolic compound concentrations were noted by other authors [Capecka et al. 2005, Cosio et al. 2006, Shan et al. 2005, Wong and Kitts 2006, Zheng and Wang 2001].

The separation of ethanol extracts into fractions I, II and III produced significant differences in phenolic compound levels (Fig. 1). In almost all of the studied plants, the highest concentrations of phenolic compounds were reported in fraction II. Statistically

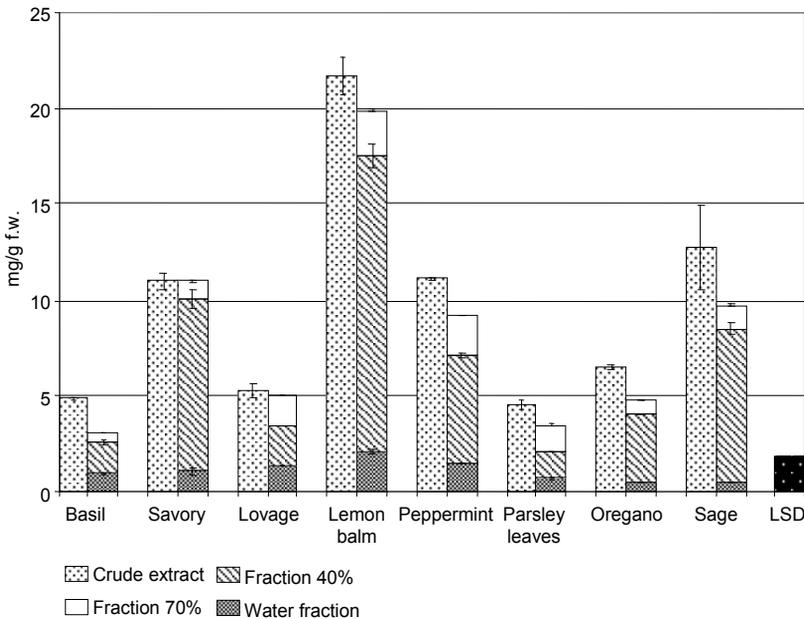


Fig. 1. Phenolic compounds content in ethanolic extracts from eight herbs (mg chlorogenic acid/g f.m.) and their diversification in fractions: water (fraction I), 40%-methanol (fraction II) and 70%-methanol (fraction III;  $\pm$ SD,  $n = 2$ )

significant differences in the total content of phenolic acids were noted between fraction II and the remaining fractions in savory, lemon balm, peppermint, oregano and sage extracts where fractions I and III contained more than 80% less phenolic compounds than fraction II (Fig. 1). Comparable quantities of phenolic compounds in fractions with increasing lipophilicity were observed in basil, lovage and parsley extracts.

There are no published studies investigating the separation of phenolic compounds into fractions with increasing lipophilicity. Wong and Kitts [2006] are the only authors who carried out two simultaneous extractions in 100% methanol and water, reporting a lower total content of phenolic compounds in the aqueous extract than in the methanol extract of parsley. In both cases, the results reported by the above authors were below the values noted in this study. The above suggests that the 80% solution offers greater versatility in isolating total phenolic compounds than pure methanol or pure water.

An analysis of the effect of the storage time of ethanol extracts on changes in the total content of phenolic compounds showed that extract components were marked by varied degree of susceptibility to oxidation and reduction during storage (Fig. 2). A significant drop in the content of phenolic compounds after three months of storage was reported in lemon balm, peppermint, parsley and sage extracts, reaching from 21.5% to 25.9%. A further decrease in the total content of phenolic compounds after the successive three months of storage was observed only in peppermint and parsley extracts which contained 50% less of the studied compounds after six months of storage (Fig. 2). The extracts from the remaining plant species were characterised by high stability, and no significant differences in the total content of phenolic compounds were noted after three and six months of storage.

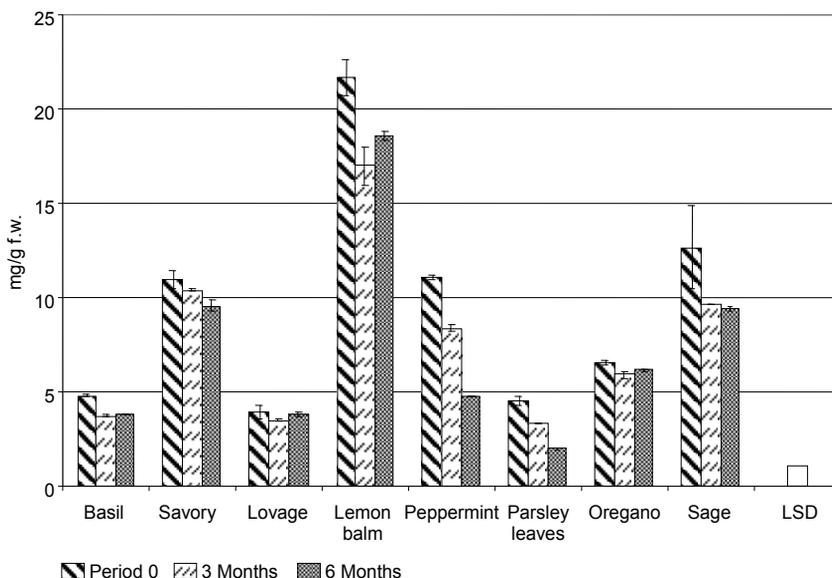


Fig. 2. Total phenols changes in ethanolic extracts (mg chlorogenic acid/g f.w.) depending on storage period ( $\pm$ SD, n = 2)

Similarly to ethanol extracts, aqueous fractions were marked by high stability throughout the period of storage (Fig. 3). The least stable aqueous fractions were obtained from parsley and lemon balm where a 40% and a 22% drop in the total content of phenolic compounds was noted after three months of storage, respectively. The highest degree of stability was demonstrated by aqueous fractions produced from savory, oregano and sage where no significant differences in the total content of phenolic compounds were observed after six months of storage.

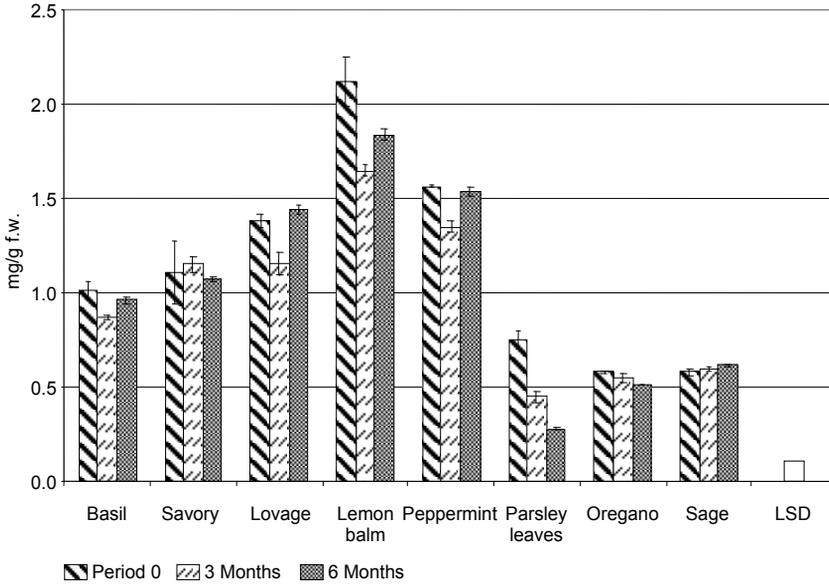


Fig. 3. Total phenols changes in fraction I (mg chlorogenic acid/g f.w.) depending on storage period ( $\pm$ SD,  $n = 2$ )

Changes in the total content of phenolic compounds in fraction II are shown in Figure 4. From among the eight plants studied, the most stable 40% methanol extracts were characterised by a low total content of phenolic compounds, i.e. fraction II from basil, lovage, peppermint and parsley. The highest drop of 42% in the total content of phenolic compounds was reported in the fraction from savory after three months of storage, as well as in fractions from lemon balm and oregano whose phenolic compound content decreased by 25% after three months of storage. A further decrease in the stability of the analysed compounds was not observed after the successive three months of storage (Fig. 4).

The stability of phenolic compounds in fraction III was not determined by their concentrations. Lovage and peppermint extracts were marked by the highest stability throughout the storage period and contained much higher levels of phenolic compounds than the remaining plants (Fig. 5). Significant changes in the total content of phenolic acids were noted in 70% methanol extracts of basil, savory, lemon balm, parsley and sage. In those extracts, the total content of phenolic acids decreased by 8.1% to 24.6% after three months of storage. No significant changes in the content of phenolic compounds were reported in any of the analysed extracts after the successive three months

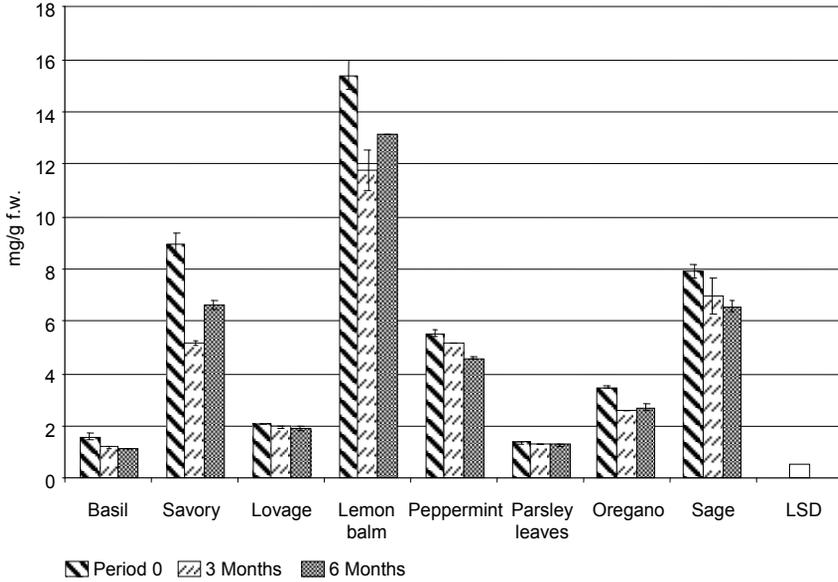


Fig. 4. Total phenols changes in fraction II (mg chlorogenic acid/g f.w.) depending on storage period ( $\pm$ SD, n = 2)

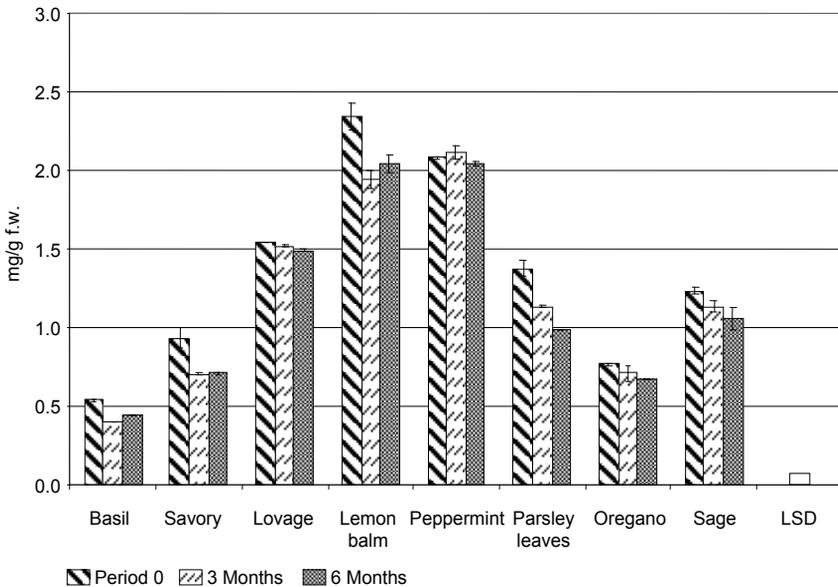


Fig. 5. Total phenols changes in fraction III (mg chlorogenic acid/g f.w.) depending on storage period ( $\pm$ SD, n = 2)

of storage, except for parsley extracts whose phenolic compound content decreased by a further 13% in comparison with the extracts stored for three months.

In most studies, the total content of phenolic compounds is usually determined directly after extract preparation to eliminate any storage loss [Areias et al. 2000, Shan et al. 2005]. Nevertheless, the loss of phenolic compounds over time cannot be avoided in herbal extracts used as dietary supplements. For this reason, this study set out to determine the stability of herbal extracts. As demonstrated by the present results, phenolic compounds found in herbal extracts are relatively stable. In most cases, following a decrease in the initial period of storage (up to three months), further storage did not have a significant effect on the degradation of phenolic compounds. At the next stage of the study, the reactivity of hydrophilic and lipophilic fractions with various oxidants will be determined to evaluate their antioxidant capacity. In-depth studies will also be carried out to investigate extract stability between 0 and 3 months of storage as most oxidation processes take place during that time.

## SUMMARY

The results of this study indicate that lemon balm is the most abundant source of phenolic compounds in the group of the studied herbal plants. Despite a substantial decrease in the total content of phenolic compounds after three months of storage (around 20%), ethanol extracts and fractions I, II and III contained the highest levels of the studied compounds. Phenolic compounds found in herbal plants are hydrophilic to lipophilic substances which are stable compounds regardless of their affinity for water. Substances marked by average lipophilicity had the highest share of the determined compounds (fraction II). This group of compounds includes flavonoid derivatives which become more hydrophilic through glycosylation of hydroxyl groups. It may also include phenolic acid derivatives whose hydrophilicity is lowered by carboxyl group esterification. Those compounds are a potent source of antioxidants used as dietary supplements.

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## OCENA LIPOFILNOŚCI ZWIĄZKÓW FENOLOWYCH I ICH TRWAŁOŚCI W EKSTRAKTACH Z ROŚLIN ZIOŁOWYCH

**Wprowadzenie.** Związki fenolowe należą do roślinnych metabolitów wtórnych, które występują w różnych miejscach komórek, zależnie od ich lipofilności. Celem pracy było określenie różnic w zawartości związków fenolowych we frakcjach z ekstraktów ziołowych o wzrastającej lipofilności oraz ich trwałości.

**Materiał i metody.** Analizie poddano osiem roślin ziołowych: bazylię, cząber, lubczyk, melisę, mięętę, natkę pietruszki, oregano i szalwię. Ekstrakty przygotowano ze świeżych roślin zbieranych pod koniec lipca, następnie rozdzielano je na frakcję wodną, metanolową – 40- i 70-procentową – na żelu krzemionkowym C18. W uzyskanych frakcjach oznaczono całkowitą zawartość związków fenolowych (metodą Folina-Ciocalteu) bezpośrednio po przygotowaniu frakcji oraz po przechowywaniu przez trzy i sześć miesięcy.

**Wyniki.** Stwierdzono, że trwałość ekstraktów przygotowanych ze świeżych ziół była różna, zależnie od gatunku rośliny. Najbogatszy w związki fenolowe ekstrakt z liści melisy wykazywał najwyższy poziom polifenoli w całym okresie przechowywania. Największy spadek zawartości związków fenolowych zaobserwowano w ekstraktach z natki pietruszki. Po rozdziale ekstraktów surowych na trzy frakcje, najwyższy poziom związków fenolowych stwierdzono we frakcjach wyizolowanych 40-procentowym roztworem metanolu. Ponadto wykazano, że niezależnie od lipofilności frakcji, najbardziej dynamiczne zmiany w ilości polifenoli zachodziły w pierwszym okresie przechowywania (3-miesięcznym). Związki, które nie uległy rozpadowi po tym czasie wykazywały dużą trwałość.

**Wnioski.** Związki fenolowe zawarte w roślinach ziołowych to substancje od hydro- do lipofilowych, które są związkami trwałymi, niezależnie od powinowactwa do wody.

**Słowa kluczowe:** zioła, związki fenolowe, przechowywanie

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