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**Essential metals and phenolic acids in commercial herbs and spices. Multivariate analysis of correlations among them**

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**Abstract:** The purpose of this study was to show relationships among the levels of essential metals (zinc, iron, sodium, magnesium, calcium and potassium) and phenolic acids (caffeic, chlorogenic, ferulic, gallic, rosmarinic and syringic) in commercial herbs (lemon balm, thyme, rosemary, mint, sage and angelica) and spices (caraway, lovage, hyssop and oregano). In the herbs higher quantities of metals and phenolic compounds were found than in spices. All plants contained high levels of calcium, potassium and rosmarinic acid, but low levels of zinc and gallic acid. By using principal component and hierarchical cluster analyses several clusters were identified grouping samples originating from a plant of a particular botanical species. Multivariate analysis has also shown that the contents of phenolic acids had a stronger impact on the scattering of herbs and spices than the metals levels. Furthermore, statistically significant correlations were found between calcium and ferulic, gallic, rosmarinic and syringic acids as well as between zinc and sodium and caffeic acid. This suggests co-operate between these biologically active constituents in metabolic processes occurring in plants.

**Keywords:** essential metals, phenolic acids, herbs, spices, multivariate analysis

**1 Introduction**

Various plants have been used since antiquity not only for their culinary benefits but also for their role played in health protection as preventive or supportive therapies of numerous diseases and disorders [1-4]. Many plants have been used as folk remedies after preparation in traditional way such as cooking, infusions or macerations. The therapeutic activity of herbs is associated with the content of biologically active elements and organic compounds of varying structures and remedial powers such as vitamins, alkaloids or tannins [3]. Some of plants have also been used as spices which play a major role in cooking, cosmetics and perfumery [5]. Culinary herbs and spices added flavour and improved the palatability of food. In the European Union, basil, bay leaves, celery, coriander, oregano, rosemary, sage and thyme are widely cultivated plants for dietary purposes [6]. At the same time, basil, lemon balm, mint, rosemary and sage are commonly used in the Mediterranean dishes. Hence, the consumption of spices all over the world constantly increases [7].

Herbs and spices constitute an important link in the transfer of essential metals from soil to man [3]. Knowledge about the level of metals in plants is important because many trace metals play a crucial role in the formation of constituents responsible for the curative and nutritional properties. Trace elements are also incorporated in the structures of proteins, enzymes, and carbohydrate complexes that participate in human biochemical reactions [8,9]. For example, zinc and iron together with enzymes are necessary for the functioning and maintenance of the immune system. Zinc is also essential for the activity of numerous enzymes, whereas iron is an essential element for living cells and its deficiency is associated with anaemia. Magnesium is present in many enzymes, it plays a major role in overall cell functions and in metabolism of proteins, lipids and carbohydrates [10]. In turn, sodium is responsible for depolarization of cellular membrane and for the water equilibrium in the intra- and extra cellular medium, while potassium maintains the fluid and electrolyte balance in the body and is responsible for sending nerve impulses [10,11]. On the other hand, calcium is the most abundant element that helps to form and maintain healthy bones [8].

Potential benefits of herbs and spices have also been attributed to the presence of phenolic compounds.

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Among them phenolic acids play a crucial role [12,13]. These compounds encompass hydroxylated derivatives of benzoic and cinnamic acids and, the most common among them being caffeic, ferulic, \( p \)-coumaric, \( p \)-hydroxybenzoic, vanilic and procatechuic acids [14,15]. Phenolic acids constitute a group of secondary metabolites with an antioxidant, metal-chelating and redox properties, acting as reducing agents, hydrogen donators or singlet oxygen quenchers [16]. These acids have also been known to exhibit various pharmacological activities such as vasoprotective, anti-carcinogenic, antiviral, anti-inflammatory and antiallergenic, which can be attributed to phenolics as antioxidants. Moreover, recent studies have shown beneficial properties of chlorogenic acid to humans, such as their antioxidant, hepatoprotective and hypoglycaemic potentials [17]. Promising gallic acid activity was found in lemon balm extracts in the treatment of Alzheimer’s disease due to its antioxidant and matrix metalloproteinase-2 inhibitory activity [18], and many biological activities of rosmarinic acid, such as inhibiting of HIV-1, antitumor, anthepatitis, and protecting the liver, inhibiting blood clots and anti-inflammation [19].

Taking these reasons into consideration, the aim of this study was to quantify the content of selected essential metals (zinc, iron, sodium, magnesium, calcium and potassium) and phenolic acids (caffeic, chlorogenic, ferulic, gallic, rosmarinic and syringic) in herbs and spices in order to learn, whether or not these plants differ significantly in their metallic and phenolic profiles, and to establish relationships between these two groups of biologically active constituents of plants. The above-mentioned metals and phenolics were examined in this study because potential health benefits of some herbs and spices have been attributed to the presence of these constituents. Selected plant species chosen for the analysis were characterised by a similarity in secondary metabolite composition. These plant materials contain crucial substances from the biological point of view, such as essential oils, terpenes and phenolic compounds, for example phenolic acids, flavonoids and tannins. Due to these specific constituents, they have commonly been used in different formulations, for instance as extracts, infusions and raw materials for medicinal and culinary purposes. The data sets acquired in this study were subjected to principal component (PCA) and hierarchical cluster (HCA) analyses as well as to Pearson’s correlation analysis to display correlations between essential metals and phenolic acids in the plants.

### 2 Experimental Procedure

#### 2.1 Samples, reagents and standards

Thirty-one samples of commercial plant materials consisting of 20 medicinal herbs and 11 spices were used in this study. These samples were obtained from herbal enterprises in Poland and are specified in Table 1, along with taxonomical and chemical characteristics of the plants from which herbs and spices originated [20-22]. Arabic digits given in the first column of Table 1 denote numbers by which herbs and spices are labelled in the text, figures and remaining tables. One sample of each plant was prepared by mixing three commercial packaging of plant raw material taken from different batches. After powdering, the samples were homogenised at 20°C for 20 s in a water-cooled grinder Knifetec 1095 (Foss Tector, Sweden).

Acetic acid and methanol, both HPLC grade, were acquired from POCh (Poland). Perhydrol (30% solution of \( \text{H}_2\text{O}_2 \), Selectipur®) and concentrated nitric acid (65% solution of \( \text{HNO}_3 \), Selectipur®) were purchased from Merck (Germany). Redistilled water was obtained by triple distillation of water in a Destamat® Bi-18 system (Heraeus Quarzglas, Germany).

Standardised solutions of zinc, iron, sodium, magnesium, calcium and potassium (1 mg mL\(^{-1} \), Titrisol®) were purchased from Merck (Germany) while the standardised phenolic acids including caffeic, chlorogenic, ferulic, gallic, rosmarinic and syringic acids were obtained from ChromaDex (USA).

#### 2.2 Analytical procedures

To quantify the essential metals, each plant sample (0.9–1.4 g) was placed in a teflon crucible. 3 mL of perhydrol and 5 mL of nitric acid were added and the sample was digested using a microwave high-pressure system UniClever™, BM-1z (Plazmatronica, Poland). The digestion was carried out in one stage at an 85% power of magnetron for 7 min and at programmed threshold pressure values. The digested sample was cooled for 10 min and then transferred into a graduated flask. The volume was made up to 50 mL with water. A Varian SpectrAA 250 Plus spectrometer (Varian, Australia) was used for the determination of zinc, iron and magnesium (F-AAS), and sodium, calcium and potassium (F-AES).
Table 1: The taxonomical, morphological and chemical characteristics of the herbs and spices under study.

| Sample number | Herbs and spices | Herbal enterprises | Plant species and botanical families | Morphological parts of plant | Principal chemical constituents [20-22] |
|---------------|------------------|--------------------|-------------------------------------|-----------------------------|----------------------------------------|
| **Medicinal herbs** |
| 1 | Lemon balm | Flos (Mokrsko) | Melissa officinalis L. Lamiaceae | leaves | 0.1–0.4% of essential oil (citral A, citral B, citronellal), phenolic acids (rosmarinic, caffeic), triterpenic acids, flavonoids |
| 2 | Kawon (Gostyn) | |  |
| 3 | Dary Natury (Koryciny) | |  |
| 4 | Thyme | Flos (Mokrsko) | Thymus vulgaris L. Lamiaceae | herbs | 0.5–2.5% of essential oil (thymol, carvacrol), tannins, phenolic acids, triterpenic acids, flavonoids |
| 5 | Kawon (Gostyn) | |  |
| 6 | Herbalux (Warsaw) | |  |
| 7 | Rosemary | Kawon (Gostyn) | Rosmarinus officinalis L. Lamiaceae | leaves | 0.4–2.4% of essential oil (terpinene, terpineol-(4), (+)-α-terpineol), tannins, flavonoids, phenolic acids (rosmarinic, caffeic, p-coumaric, ferulic) |
| 8 | Flos (Mokrsko) | |  |
| 9 | Herbalux (Warsaw) | |  |
| 10 | Kawon (Gostyn) | |  |
| 11 | Mint | Flos (Mokrsko) | Mentha piperita L. Lamiaceae | leaves | 1.0–3.0% of essential oil ((–)-menthol, acetic and valeric eaters of menthol), tannins, phenolic acids |
| 12 | Herbapol (Krakow) | |  |
| 13 | Herbapol (Lublin) | |  |
| 14 | Kawon (Gostyn) | |  |
| 15 | Dary Natury (Koryciny) | |  |
| 16 | Sage | Flos (Mokrsko) | Salvia officinalis L. Lamiaceae | leaves | up to 3.0% of essential oil (tujone, cneyl, camphor), bitter diterpenic lactones, triterpenic acids, phenolic acids (rosmarinic, caffeic) |
| 17 | Herbalux (Warsaw) | |  |
| 18 | Kawon (Gostyn) | |  |
| 19 | Dary Natury (Koryciny) | |  |
| 20 | Angelica | Kawon (Gostyn) | Archangelica officinalis Hoffm. Apiaceae | roots | up to 1.5% of essential oil (β-phellandrene, α-pinene), coumarines, flavonoids, phenolic acids (chlorogenic, caffeic) |
| **Spices** |
| 21 | Caraway | Flos (Mokrsko) | Carum carvi L. Apiaceae | fruits | 3–7% of essential oil (D(+)-carvone, D(–)-limonene), flavonoids, phenolic acids (caffeic, chlorogenic) |
| 22 | Kawon (Gostyn) | |  |
| 23 | Kawon (Gostyn) | |  |
| 24 | Lovage | Kawon (Gostyn) | Levisticum officinale Koch. Apiaceae | roots | 0.6–1.0% of essential oil (phthalides, α-pinene, β-pinene), coumarines, phenolic acids (chlorogenic, caffeic, ferulic), organic acids |
| 25 | Flos (Mokrsko) | |  |
| 26 | Herbapol (Krakow) | |  |
| 27 | Kawon (Gostyn) | |  |
| 28 | Hyssop | Kawon (Gostyn) | Hyssopus officinalis L. Lamiaceae | herbs | ~ 1% of essential oil (pinene, pinocamphene), flavonoids, phenolic acids (rosmarinic, caffeic) |
| 29 | Ziolowy Zakatek (Koryciny) | |  |
| 30 | Oregano | Dary Natury (Koryciny) | Origanum vulgare L. Lamiaceae | leaves and flowers | 0.12–1.20% of essential oil (carvacrol, thymol), phenolic acids |
| 31 | Kamis (Stefanowo) | |  |

1) Herbs are a raw plant material consisting of entire plant organism without woody and underground parts.
To quantify phenolic acids, a powdered sample (0.25 g) was sonicated in 3 mL of a mixture of methanol and water (80:20, v/v) at room temperature for 60 min. Then the sample was centrifuged at 20,000 rpm for 20 min (Hettich, Germany). Then the supernatant was transferred into a 10-mL graduated flask, this procedure was repeated in triplicate and the extracts were combined. Prior to HPLC analysis, the extracts were filtered through a 0.2 µm nylon membrane filter (Witko, Poland).

A Hitachi LaChrome HPLC system with UV-Vis detector L-7420, autosampler L-7200 and thermostat L-7360 (Merck, Germany) was used for analysis. Chromatographic data were collected using a D-7000 HPLC System Manager, ver. 3.1. The chromatographic separation and analysis were performed using a Hypersil Gold C18 column (5 µm particles, 250 mm × 4.6 mm, Thermo Scientific, UK) and a mobile phase at a flow rate of 1.0 mL min⁻¹. The mobile phase was a mixture of methanol and water (25:75, v/v) containing 1% of acetic acid. The column temperature was maintained at 25°C. Gallic and syringic acids were detected at 280 nm, whereas caffeic, chlorogenic, ferulic and rosmarinic acids at 320 nm. All injection volumes of the standard and sample solutions were 20 µL. The total time of analysis was 25 min.

### 3 Results and Discussion

#### 3.1 Methods validation

According to the requirements of ICH-Q2 [23], the basic validation parameters for procedures developed for determination of metals and phenolics are summarized in Table 2. Twelve calibration curves were generated based on a linear equation \( y = ax + b \), where \( c \) is the concentration of analyte and \( y \) is the absorbance/emission for essential metals or the area under HPLC peak for phenolic acids. Analysis of each of six independent standard solutions was performed in six replications. A good linearity as well as high correlation \((R)\) and determination \((R^2)\) coefficients were obtained for all the analytes under study over a wide concentration range. The \(R^2\) values for metals and phenolics were greater than 0.990 and 0.998, respectively. The intercept values \((b)\) for the linear equation were statistically insignificant.

Based on the slope \((a)\) and the standard error of residuals \((S_{xy})\) of the linear equation, the limits of detection (LOD) and quantification (LOQ) for all the analytes were calculated from the formulas: 3.3 \( S_{xy} a \) and 10 \( S_{xy} a \), respectively. The LOD and LOQ values fell in the ranges from 0.07 to 0.47 µg mL⁻¹ and from 0.12 to 1.43 µg mL⁻¹, respectively for metals, and for phenolics from 0.49 to 27.55 µg mL⁻¹ and from 1.49 to 83.49 µg mL⁻¹, respectively. These values showed that the sensitivity of the methods was satisfactory.

The precision (expressed as coefficient of variation) and accuracy (expressed as recovery) for all the analytes were determined by six replicate analyses of standard solutions. The precision and recovery levels were respectively: 2.41–16.39% and 83.67–103.75% for essential metals, and 0.34–5.96% and 97.87–102.59% for phenolic acids. Thus, the developed methods provide simple, rapid and reliable analytical procedures for the determination of metals and phenolics.

#### 3.2 Metals and phenolics in plants

As shown in Table 1, thirty-one herbs and spices originating from two botanical families were analysed. With the exception of angelica, caraway and lovage spices obtained from plants belonging to the Apiaceae family, the remaining group of plants were from the Lamiaceae family. The samples representing different morphological parts of the plants – leaves, flowers, fruits, roots and herbs (a whole aerial plant without woody parts), originated from ten plant species [20-22]. These morphological parts...
have been recommended by pharmacopoeias and culinary
guidebooks as those including higher quantities of bioactive
constituents than other tissues of the particular plant,
guaranteeing the curative and nutritive efficiency of herbs
and spices. The majority of the materials were represented
by at least three samples acquired from different herbal
enterprises, while hyssop and oregano spices merely by
two, and angelica roots, by one sample.

The results of the determination of trace elements (zinc,
iron, sodium) and macroelements (magnesium, calcium,
potassium) in the herbs and spices are compiled in Table 3.
Based on these data it was found that medicinal herbs were
richer in the metals than spices. Student’s t-test reveals that
with the exception of two pairs of elements, iron-sodium
and calcium-potassium, the differences between the levels
of other essential metals were statistically significant.

Of all the trace metals determined, sodium was
detected in the highest quantity in both herbs and spices.
In the group of herbs, the highest level of sodium was
found in the leaves of thyme (4), rosemary (7-9) and mint
(11-14), above 200 µg g⁻¹. Among the spices, four samples of
lovage (24-27) were richest in this element, above 150 µg g⁻¹.
In contrast to sodium, zinc and iron were at the lowest
quantities in the studied raw plants. The content of these
trace metals ranged between 5.92−83.35 µg g⁻¹ (zinc) and
4.22−260.74 µg g⁻¹ (iron) for herbs, and 19.00−48.41 µg g⁻¹
(zinc) and 18.77 −252.43 µg g⁻¹ (iron) for spices.

The level of magnesium in herbs and spices is higher
than that in trace elements. The leaves of mint showed
the highest value of this element (11.16 mg g⁻¹, sample 14)
among the herbs. In spices, the highest value (5.07 mg g⁻¹,
sample 23) was found in the caraway. Calcium and
potassium are the most important macroelements from the
biological point of view found in plants [8,10,11]. Among
the herbs, the highest level of this metal was determined
in the leaves of sage (31.50 mg g⁻¹, sample 19). In the

| Table 2: Validation of the procedures for determination of essential metals and phenolic acids in the herbs and spices. |
|----------------------------------------------------------|
| Essential metals | Zinc | Iron | Sodium | Magnesium | Calcium | Potassium |
| Range [µg mL⁻¹] | 0.1–1.6 | 1.0–5.0 | 0.3–2.4 | 0.1–0.5 | 0.4–3.2 | 0.3–2.4 |
| Slope a | 0.4081 | 0.1053 | 0.2977 | 1.1060 | 0.1716 | 0.3283 |
| Confidence interval of slope | 0.0195 | 0.0123 | 0.0225 | 0.1128 | 0.0129 | 0.0490 |
| Intercept b | 0.0065 | -0.0381 | 0.0500 | 0.0590 | 0.1458 | 0.0987 |
| Confidence interval of intercept | 0.0189 | 0.0408 | 0.0392 | 0.0374 | 0.0235 | 0.0787 |
| Linearity R² | 0.9995 | 0.9938 | 0.9983 | 0.9953 | 0.9974 | 0.9900 |
| LOD [µg mL⁻¹] | 0.07 | 0.47 | 0.15 | 0.04 | 0.21 | 0.32 |
| LOQ [µg mL⁻¹] | 0.23 | 1.43 | 0.46 | 0.12 | 0.65 | 0.97 |
| Coefficient of variation [%] | 2.41 | 5.97 | 2.92 | 11.06 | 16.39 | 3.28 |
| Recovery [%] | 103.75 | 83.67 | 96.00 | 103.33 | 101.67 | 98.67 |
| Phenolic acids | | | | | | |
| Caffeic acid | Chlorogenic acid | Ferulic acid | Gallic acid | Rosmarinic acid | Syringic acid |
| Range [µg mL⁻¹] | 0.16–100 | 0.16–100 | 0.16–100 | 0.16–100 | 0.16–100 |
| Slope a | 85900 | 45110 | 82748 | 42176 | 9813 | 44989 |
| Confidence interval of slope | 3070 | 2036 | 709 | 1501 | 712 | 1676 |
| Intercept b | 30025 | -52707 | -2436 | -34402 | 308221 | 15298 |
| Confidence interval of intercept | 198800 | 123251 | 26576 | 90905 | 222363 | 101515 |
| Linearity R² | 0.9993 | 0.9988 | 0.9999 | 0.9992 | 0.9992 | 0.9992 |
| LOD [µg mL⁻¹] | 3.83 | 4.83 | 0.49 | 3.81 | 27.55 | 3.99 |
| LOQ [µg mL⁻¹] | 11.62 | 14.67 | 1.49 | 11.57 | 83.49 | 12.11 |
| Coefficient of variation [%] | 2.36 | 1.62 | 5.96 | 0.34 | 1.25 | 1.98 |
| Recovery [%] | 97.95 | 100.41 | 100.03 | 97.87 | 102.59 | 99.74 |
Table 3: The content of zinc, iron and sodium in µg g⁻¹ and magnesium, calcium and potassium in mg g⁻¹ of dry weight in the herbs and spices.

| Sample number | Zinc    | Iron    | Sodium  | Magnesium | Calcium | Potassium |
|---------------|---------|---------|---------|-----------|---------|-----------|
| 1             | 36.5 ± 0.6 | 23.2 ± 0.9 | 20.4 ± 0.5 | 3.3 ± 0.1 | 11.1 ± 0.8 | 21.2 ± 0.1 |
| 2             | 29.3 ± 0.5 | 70.8 ± 4.4 | 41.3 ± 3.7 | 6.2 ± 0.2 | 24.0 ± 1.4 | 32.6 ± 1.8 |
| 3             | 20.3 ± 0.5 | 43.2 ± 2.5 | 62.1 ± 4.9 | 5.3 ± 0.5 | 19.0 ± 1.9 | 11.7 ± 0.3 |
| 4             | 14.5 ± 0.5 | 165.6 ± 2.6 | 206.6 ± 1.0 | 1.8 ± 0.1 | 6.8 ± 0.7 | 12.1 ± 0.3 |
| 5             | 34.6 ± 0.3 | 189.8 ± 6.3 | 72.7 ± 0.5 | 5.5 ± 0.5 | 12.9 ± 0.8 | 25.0 ± 5.3 |
| 6             | 35.2 ± 0.4 | 155.1 ± 4.8 | 37.5 ± 4.6 | 3.7 ± 2.2 | 24.8 ± 5.3 | 7.7 ± 4.8 |
| 7             | 9.9 ± 0.7 | 29.9 ± 1.9 | 398.8 ± 2.9 | 1.3 ± 0.2 | 10.4 ± 0.5 | 7.8 ± 0.5 |
| 8             | 17.1 ± 0.7 | 44.5 ± 1.0 | 256.1 ± 3.1 | 2.1 ± 0.1 | 14.6 ± 0.7 | 6.9 ± 0.1 |
| 9             | 8.04 ± 0.6 | 47.6 ± 1.3 | 447.3 ± 3.1 | 1.5 ± 0.1 | 3.3 ± 0.2 | 5.8 ± 0.2 |
| 10            | 13.9 ± 0.8 | 191.5 ± 6.7 | 86.3 ± 6.5 | 2.8 ± 0.1 | 21.0 ± 0.3 | 15.4 ± 1.7 |
| 11            | 12.7 ± 0.4 | 23.7 ± 0.5 | 200.4 ± 0.2 | 3.1 ± 0.1 | 11.5 ± 0.3 | 24.4 ± 0.6 |
| 12            | 23.7 ± 0.3 | 19.9 ± 1.3 | 329.6 ± 2.0 | 2.6 ± 0.1 | 11.9 ± 0.8 | 18.5 ± 0.2 |
| 13            | 5.9 ± 0.5 | 4.2 ± 0.5 | 229.3 ± 2.5 | 2.7 ± 0.1 | 9.2 ± 0.5 | 24.7 ± 0.2 |
| 14            | 17.8 ± 0.8 | 108.5 ± 2.4 | 155.9 ± 8.8 | 11.2 ± 2.1 | 16.7 ± 3.7 | 6.1 ± 1.4 |
| 15            | 29.6 ± 0.8 | 100.6 ± 1.1 | 63.2 ± 0.8 | 6.1 ± 0.3 | 23.5 ± 2.8 | 24.9 ± 7.7 |
| 16            | 6.0 ± 0.3 | 5.4 ± 0.4 | 35.2 ± 1.1 | 3.2 ± 0.2 | 11.8 ± 0.6 | 21.7 ± 0.1 |
| 17            | 25.0 ± 0.6 | 9.6 ± 0.5 | 21.1 ± 0.9 | 3.0 ± 0.1 | 11.8 ± 0.7 | 23.4 ± 0.3 |
| 18            | 83.4 ± 2.2 | 260.7 ± 16.2 | 57.3 ± 5.4 | 7.0 ± 0.7 | 22.4 ± 1.0 | 10.6 ± 1.3 |
| 19            | 24.9 ± 0.7 | 49.0 ± 2.2 | 39.8 ± 4.1 | 3.7 ± 2.3 | 31.5 ± 2.9 | 8.2 ± 6.7 |
| 20            | 31.4 ± 1.3 | 258.9 ± 19.2 | 57.6 ± 3.5 | 2.2 ± 0.1 | 3.1 ± 0.2 | 29.4 ± 2.3 |
| 21            | 35.5 ± 0.8 | 57.1 ± 1.0 | 4.9 ± 0.1 | 2.3 ± 0.1 | 7.4 ± 0.1 | 10.0 ± 0.3 |
| 22            | 27.9 ± 1.8 | 18.8 ± 0.3 | 17.3 ± 1.4 | 3.0 ± 0.2 | 9.2 ± 1.8 | 17.9 ± 4.8 |
| 23            | 48.4 ± 3.5 | 30.0 ± 2.4 | 47.8 ± 2.7 | 5.1 ± 0.2 | 16.1 ± 0.8 | 10.4 ± 0.2 |
| 24            | 23.6 ± 0.9 | 252.4 ± 4.4 | 267.4 ± 6.5 | 1.0 ± 0.2 | 2.0 ± 0.5 | 12.4 ± 0.5 |
| 25            | 19.0 ± 0.9 | 165.4 ± 5.0 | 163.2 ± 6.8 | 1.5 ± 0.1 | 5.0 ± 0.9 | 16.5 ± 1.2 |
| 26            | 25.6 ± 2.5 | 44.7 ± 3.8 | 470.9 ± 1.2 | 2.6 ± 0.2 | 6.7 ± 1.6 | 26.8 ± 2.0 |
| 27            | 19.3 ± 0.5 | 222.3 ± 6.0 | 259.8 ± 8.6 | 2.0 ± 0.1 | 4.2 ± 0.9 | 14.9 ± 3.0 |
| 28            | 27.0 ± 0.6 | 97.6 ± 2.5 | 31.2 ± 0.6 | 1.6 ± 0.1 | 9.9 ± 0.7 | 18.8 ± 0.5 |
| 29            | 24.4 ± 1.0 | 50.2 ± 1.1 | 52.3 ± 0.8 | 1.2 ± 0.1 | 8.6 ± 0.7 | 21.7 ± 0.3 |
| 30            | 27.1 ± 0.6 | 141.1 ± 3.8 | 105.5 ± 0.4 | 1.7 ± 0.1 | 15.1 ± 0.5 | 14.2 ± 0.7 |
| 31            | 24.7 ± 0.3 | 99.8 ± 2.4 | 56.5 ± 1.9 | 2.1 ± 0.1 | 15.7 ± 0.8 | 13.4 ± 0.5 |
spices, caraway showed the highest level of the element (16.05 mg g\(^{-1}\), sample 23). A literature screening shows that these values are higher than the calcium level found in herbs and spices from Turkey [24].

The most abundant mineral constituent in herbs and spices was potassium. This element is an essential dietary nutrient. It constitutes about 70% of the cations found in the plant and human cells [8]. Among herbs, the highest potassium amount was found in the leaves of lemon balm (32.57 mg g\(^{-1}\), sample 2) while the lowest in the leaves of rosemary (5.79 mg g\(^{-1}\), sample 9). Similarly, the highest and lowest quantities of potassium in spices were determined as 26.75 mg g\(^{-1}\) in lovage (sample 26) and 9.96 mg g\(^{-1}\) in caraway (sample 21). To sum up, lovage herbs and spices of thyme seemed to be the most abundant in mineral constituents.

The results of determination of caffeic, chlorogenic, ferulic, gallic, rosmarinic and syringic acids in the herbs and spices are compiled in Table 4. In general, similarly as with essential metals, herbs are richer in phenolics than spices. Among medicinal herbs, sage leaves and thyme herbs are the richest in phenolic acids. Among spices, lovage was richest in phenolics while caraway was poorest. Student’s t-test reveals that with the exception of chlorogenic acid, the mean content of gallic and rosmarinic acids in herbs and spices differ significantly from that determined for the remaining phenolics.

One of the phenolics commonly found in the plant kingdom is rosmarinic acid. A screening of the literature shows that this acid is characterised by different biological activities [19,25]. This secondary metabolite was found in plants under study at the highest quantity, varying between 0.19 and 26.45 mg g\(^{-1}\) and 0.09 and 3.32 mg g\(^{-1}\) for herbs and spices, respectively. Its highest content was found in the herb of thyme (sample 6). Moreover, all the leaves of sage were also very rich in rosmarinic acid.

As well as rosmarinic acid, the plants were also rich in chlorogenic acid. The highest quantity of this acid varying within the range of 8.20 to 18.22 mg g\(^{-1}\) was found in the thyme herbs (samples 4-6). Among the studied plants, the lowest levels of chlorogenic acid were found in the leaves of mint (0.09 mg g\(^{-1}\), sample 12) and in caraway spices (0.21 mg g\(^{-1}\), sample 21). All the leaves of rosemary (samples 7-10) were rich in caffeic acid while among the spices lovage was the richest in this acid (0.61 mg g\(^{-1}\), sample 25). Caraway (samples 21-23) was the poorest not only in caffeic acid, but also in ferulic acid. The lowest content of the latter (0.01 mg g\(^{-1}\)) was found both in herbs and spices. In two samples of caraway (samples 21 and 23) ferulic acid was not detected because it was below the LOD.

The level of syringic acid in herbs was higher than that in spices. It was found in the highest quantity in the leaves of sage (samples 16-19, 3.25-6.09 mg g\(^{-1}\)), whereas among the spices, caraway (samples 21-23) was poorest in this acid. Gallic acid was determined in the lowest quantities. Its average content varied from 0.02 to 0.36 mg g\(^{-1}\) and from 0.01 to 0.20 mg g\(^{-1}\) in herbs and spices, respectively.

In general, these findings support characteristics of phenolic acids occurrence in plants as shown in the last column of Table 1. Excluding thyme herbs, mint leaves and oregano spices, rosmarinic and caffeic acids are the most abundant phenolics in the remaining plants from the Lamiaceae family. The highest quantities of rosmarinic and caffeic acids were found in the leaves of sage (~ 20 mg g\(^{-1}\)) and rosemary (~ 5.6 mg g\(^{-1}\)). These results are compatible with those reported by Bandoni et al. [26] for rosmarinic acid in Salvia officinalis (19.5 mg g\(^{-1}\)) and by Vallverdú-Queralt et al. [27] for caffeic acid in rosemary. Furthermore, chlorogenic acid is the most abundant in the plants from the Apiaceae family. Its highest levels were found in lovage spices (~ 4.3 mg g\(^{-1}\)) and angelica roots (~ 2.4 mg g\(^{-1}\)). Caraway includes this acid in the highest quantity among all the analysed phenolics in this sample. A higher content of chlorogenic acid in caraway (4.7 mg g\(^{-1}\)) has been reported by Pandey et al. [28]. Moreover, the quantities of caffeic acid are comparable for the majority of plants from both botanical families.

### 3.3 Multivariate analysis

To disclose the relations between essential metals and phenolic acids in the plants under study, a multivariate approach based on principal component (PCA) and hierarchical cluster (HCA) analyses was used [29].

The results of PCA for the content of essential metals (Table 3) show that three principal components (PCs) with eigenvalues higher than one were obtained. Because two first PCs, PC1 and PC2 explained more than 57% of the variability, a PCA score plot can be presented in the form of a two dimensional plane. Sodium content in the plants (positively correlated to PC1 axis) and zinc, magnesium and calcium (negatively correlated) affected the distribution of samples along the PC1 axis while iron was the discrimination factor along the PC2 axis. Hence, the plants are grouped in Fig. 1a according to similarities in their metallic profiles. For example, the samples richest in sodium are localised on the right-hand side of the PCA plot (cluster II) while those richest in iron on the left-hand side (cluster III). Further, almost all the spices (denoted by a circle) are found in the central part of the PCA plot.

For the content of phenolic acids (Table 4), two PCs were obtained with eigenvalues higher than one that explained
Table 4: The content of phenolic acids in mg g\(^{-1}\) of dry weight in the herbs and spices.

| Sample number | Caffeic acid  | Chlorogenic acid | Ferulic acid  | Gallic acid  | Rosmarinic acid | Syringic acid |
|---------------|---------------|------------------|---------------|-------------|----------------|--------------|
| 1             | 0.264 ± 0.001 | 0.142 ± 0.001    | 0.063 ± 0.001 | 0.211 ± 0.001 | 7.483 ± 0.001  | 0.091 ± 0.001 |
| 2             | 0.591 ± 0.001 | 0.285 ± 0.001    | 0.022 ± 0.001 | 0.365 ± 0.001 | 1.851 ± 0.001  | 0.950 ± 0.001 |
| 3             | 0.762 ± 0.001 | 0.212 ± 0.001    | 0.098 ± 0.001 | 0.144 ± 0.001 | 14.061 ± 0.001 | 0.244 ± 0.002 |
| 4             | 0.775 ± 0.001 | 8.201 ± 0.008    | 0.362 ± 0.001 | ND          | 7.608 ± 0.001  | 0.619 ± 0.006 |
| 5             | 1.370 ± 0.001 | 18.221 ± 0.001   | 0.249 ± 0.001 | 0.249 ± 0.001 | 10.930 ± 0.001 | 0.740 ± 0.002 |
| 6             | ND            | 14.800 ± 0.012   | 0.073 ± 0.001 | ND          | 26.450 ± 0.013 | 0.818 ± 0.007 |
| 7             | 4.131 ± 0.001 | 0.762 ± 0.002    | 0.115 ± 0.001 | 0.234 ± 0.001 | 2.324 ± 0.002  | 0.825 ± 0.005 |
| 8             | 5.082 ± 0.001 | 1.891 ± 0.003    | 0.151 ± 0.001 | 0.149 ± 0.001 | 3.675 ± 0.002  | 0.362 ± 0.001 |
| 9             | 6.595 ± 0.001 | 1.984 ± 0.005    | 0.051 ± 0.001 | 0.149 ± 0.001 | 1.343 ± 0.001  | 1.080 ± 0.003 |
| 10            | 6.417 ± 0.001 | 3.675 ± 0.003    | 0.035 ± 0.001 | 0.203 ± 0.001 | 1.343 ± 0.001  | 1.423 ± 0.001 |
| 11            | 0.060 ± 0.001 | 0.581 ± 0.001    | 0.900 ± 0.001 | 0.21 ± 0.001  | 4.324 ± 0.001  | 0.468 ± 0.001 |
| 12            | 0.043 ± 0.001 | 0.094 ± 0.002    | 0.620 ± 0.001 | ND          | 5.977 ± 0.001  | 0.223 ± 0.001 |
| 13            | 0.110 ± 0.001 | 0.232 ± 0.001    | 0.507 ± 0.001 | 0.091 ± 0.001 | 4.819 ± 0.001  | 0.315 ± 0.001 |
| 14            | 0.122 ± 0.001 | 0.242 ± 0.001    | 0.671 ± 0.001 | 0.033 ± 0.001 | 6.906 ± 0.001  | 0.218 ± 0.001 |
| 15            | 0.029 ± 0.001 | 0.541 ± 0.001    | 0.223 ± 0.001 | 0.062 ± 0.001 | 3.145 ± 0.001  | 0.568 ± 0.001 |
| 16            | 2.091 ± 0.001 | 2.784 ± 0.004    | 0.229 ± 0.001 | 0.342 ± 0.001 | 21.203 ± 0.002 | 5.972 ± 0.002 |
| 17            | 1.056 ± 0.002 | 2.094 ± 0.002    | 0.227 ± 0.001 | 0.214 ± 0.001 | 23.426 ± 0.016 | 4.985 ± 0.001 |
| 18            | 0.851 ± 0.001 | 1.653 ± 0.003    | 0.460 ± 0.008 | 0.308 ± 0.001 | 25.527 ± 0.002 | 3.255 ± 0.001 |
| 19            | 1.622 ± 0.001 | 1.983 ± 0.002    | 0.010 ± 0.002 | 0.291 ± 0.001 | 13.162 ± 0.001 | 6.090 ± 0.008 |
| 20            | 0.077 ± 0.008 | 2.373 ± 0.002    | 0.127 ± 0.007 | 0.020 ± 0.003 | 1.88 ± 0.003   | 0.600 ± 0.006 |
| 21            | 0.022 ± 0.007 | 0.205 ± 0.002    | ND            | ND          | ND             | ND           |
| 22            | ND            | 0.871 ± 0.004    | 0.011 ± 0.006 | 0.150 ± 0.008 | 0.094 ± 0.001  | 0.065 ± 0.005 |
| 23            | 0.032 ± 0.003 | 0.848 ± 0.001    | ND            | 0.200 ± 0.001 | 1.680 ± 0.002  | 0.201 ± 0.001 |
| 24            | 0.040 ± 0.002 | 4.040 ± 0.002    | 0.619 ± 0.002 | 0.051 ± 0.002 | 3.323 ± 0.006  | 0.133 ± 0.002 |
| 25            | 0.609 ± 0.001 | 4.900 ± 0.003    | 3.851 ± 0.004 | 0.081 ± 0.006 | 2.728 ± 0.005  | 0.105 ± 0.006 |
| 26            | 0.551 ± 0.002 | 3.237 ± 0.002    | 1.962 ± 0.001 | 0.071 ± 0.003 | 2.444 ± 0.008  | 0.155 ± 0.005 |
| 27            | 0.551 ± 0.004 | 4.976 ± 0.002    | 0.977 ± 0.002 | 0.039 ± 0.002 | 3.294 ± 0.004  | 0.710 ± 0.004 |
| 28            | 0.393 ± 0.006 | 2.711 ± 0.001    | 0.562 ± 0.002 | 0.071 ± 0.003 | 3.031 ± 0.005  | 0.483 ± 0.002 |
| 29            | 0.512 ± 0.002 | 2.090 ± 0.008    | 0.872 ± 0.004 | ND          | 9.151 ± 0.003  | 0.712 ± 0.006 |
| 30            | 0.276 ± 0.001 | 0.984 ± 0.001    | 0.091 ± 0.001 | 0.021 ± 0.004 | 0.391 ± 0.001  | 0.253 ± 0.002 |
| 31            | 0.091 ± 0.001 | 0.786 ± 0.001    | 0.222 ± 0.001 | 0.011 ± 0.003 | 0.782 ± 0.001  | 0.342 ± 0.005 |

**ND** – not detected.
more than 65% of the variability. Distribution of herbs and spices in a PCA score plot (Fig. 1b) is quite different compared to that representing essential metals (Fig. 1a). This can be due to the fact that phenolics are secondary metabolites encompassing a large group of naturally occurring compounds characteristic of a particular botanical species [30]. Thus, Fig. 1b shows that the herb of thyme (sample 6) is distinctly separated from the others because of the highest rosmarinic acid level among the remaining samples and high content of chlorogenic acid, whereas caffeic and gallic acids were not detected in this herb. The leaves of sage (samples 16-19), which in comparison to the other plants are characterised by the highest contents of gallic, rosmarinic and syringic acids and a high content of chlorogenic acid, whereas caffeic and gallic acids were not detected in this herb. The leaves of rosemary (samples 7-10) are grouped in cluster B, while the leaves of lemon balm (samples 1 and 3) distinguished by a high level of rosmarinic acid are found in cluster C. Because of the lower level of rosmarinic acid, the third sample of lemon balm (sample 2) was shifted into cluster B. Moreover, two samples of lovage spice (25, 26) form cluster D. Thus, PCA enables identification of several clusters grouping samples originating from plants of the same botanical species. The gallic, rosmarinic and syringic acids contents in the plants had the strong impact on classification of the samples along the PC1 axis (negatively correlated to this axis) while also negatively correlated chlorogenic and rosmarinic acids were the predominant factors for classification of the samples along the PC2 axis.

The most interesting scattering of the plant samples was obtained for pooled data (Tables 3 and 4). Out of five PCs with eigenvalues higher than one, PC1 and PC2 explained more than 44% of the variability. Sodium content in the samples (positively correlated to PC1), and rosmarinic acid and calcium (negatively correlated) had an impact on the distribution of the samples along the PC1 axis while caffeic and gallic acids were discriminating factors of PC2 axis. The sample localization shown in Fig. 1c displayed some characteristic groups of herbs and spices, similarly as in the case of PCA for phenolics (Fig. 1b). The outlying sample 6 is distinctly separated from the others, whereas all the leaves of rosemary and sage and lovage spice create clusters A, B and C, respectively. All the samples (1-3) of the lemon balm are grouped between clusters A and B, while the leaves of mint (samples 11-13) are located between clusters B and C. These findings show that the contents of phenolics had a stronger impact on distribution of the herbs and spices than the contents of metals. Phenolic acids enable grouping of the samples originating from plants of a particular botanical species into separate clusters.

The next multivariate approach, HCA, confirms the results obtained by PCA indicating the same characteristic groups of plant samples. As presented in Fig. 2a, four clusters are displayed at the linkage distance of 33% of its maximum length, which group the plants according to similarities in their metallic profile. Cluster I is created by 6 out of 11 samples of spices, including caraway and hyssop. In this cluster there are also all of the lemon balm leaves and three out of the four leaves of sage. Inspection of the data listed in Table 3 shows large variations in the essential metals levels in the herbs and spices creating this cluster. Regardless of that, after exclusion of caraway (samples 21-23), cluster I groups solely raw plant samples from the Lamiaceae family. The remaining clusters are more interesting because they are created by the samples with either the highest or the lowest level of the metals. The samples richest in sodium are included in cluster II while those in iron fall in cluster III. Herbs and spices grouped in cluster IV are distinguished by their high levels of iron and sodium as compared to those of the remaining samples.
The results of HCA for phenolic acids (Table 4) are illustrated in Fig. 2b. Inspection of this graph shows that the HCA dendrogram confirms the results obtained by PCA (Fig. 1b) indicating the same characteristic groups of samples. There are three clusters at the linkage distance of 33% of its maximum length. Cluster I creates the thyme herb only (sample 6). Cluster II encompasses leaves of sage (samples 16-18). The fourth sample of sage (sample 19) was included into cluster III because of the lower content of rosmarinic acid. This large cluster is created by 27 samples, which could be divided into two subclusters. Apart from sample 29, subcluster A consists solely of herbs, especially the leaves of lemon balm (1 and 3) and mint (samples 11-14) owing to the similarity in their phenolics composition, including chlorogenic, rosmarinic and syringic acids. However, at the lower linkage distance of approximately 10%, two groups of samples are identified in subcluster B. The first one consists of the leaves of rosemary (samples 7-10), which are characterised by the highest level of caffeic acid among the remaining samples and a high content of chlorogenic and gallic acids. The other includes mainly spices, 10 out of 11 samples of spices are grouped in this part of subcluster B.

The HCA dendrogram based on the data from Tables 3 and 4 shows scattering of the herbs and spices in Fig. 2c almost in such a way as in the case of HCA based solely on metals levels (Fig. 2a). This multivariate analysis reveals that a stronger impact on the sample discrimination have metallic profiles of the plants than the level of phenolic acids.

### 3.4 Pearson's correlation analysis

In general, curative and aromatic properties of plants depend first and foremost on the levels of secondary metabolites synthesised during normal plant development in response to stress conditions, among others wounding and UV radiation [13]. However, the biosynthesis of these constituents is not well understood. Because the herbs and spices under study originated from plants rich in the phenolic compounds (phenolic acids, flavonoids, tannins), an interesting aspect of this study was to gain a better knowledge about complex relationships between chemical components in plants (also minerals) and phenolic acids synthesised in the shikimic acid pathway. It seems that correlation analysis could be used for tentative examination of these relations. As reported in the literature, calcium stimulates the biosynthesis of caffeic and chlorogenic acids in potato and increases the levels of enzymes involved in plant defence mechanisms [31]. It will thus be beneficial for potato growers to supplement the soil with calcium to reduce the maceration effect of pectolytic pathogens, since calcium improves tuber resistance against the pathogens.

Listed in Table 5 the correlation coefficients between the levels of essential metals and phenolic acids in plants under study show that their values are statistically significant ($p < 0.05$) for 14 pairs of constituents. More significant correlations were found between the metals (5 pairs) than between the phenolics (3 pairs). In the latter case, the highest correlation coefficients were found between chlorogenic and rosmarinic acids ($r = 0.90$, Fig. 3a), and syringic and gallic acids ($r = 0.60$). The chlorogenic and rosmarinic acids occur in the plants under study in higher quantities as compared to those of the remaining phenolic acids. Literature screening also shows that occurrence of chlorogenic acid is mostly coupled with rosmarinic acid [32]. For instance, within the genus *Maranta* all species...
containing rosmarinic acid also showed the presence of chlorogenic acid. Furthermore, the biosynthesis of these acids is partially identical.

As mentioned above, phenolic compounds and related substances are synthesized via the shikimic acid pathway which occurs in plant respiration [13]. This pathway and subsequent reactions are also the source of some phenolic acids, such as cinnamic, p-coumaric, caffeic, ferulic, chlorogenic, protocatechuic and gallic acids. Hence, significant correlations between phenolics in the herbs and spices can be related to similar multistep route used by plants for the biosynthesis of phenolic acids. On the other hand, strong negative correlations were found between sodium and some metals, especially calcium (r = -0.45) and zinc (r = -0.42). This suggests negative associations between these metals in metabolic pathways. The literature screening has shown that there is no information about the reasons for which these elements are negatively correlated.

Interesting correlations were obtained for essential metals and phenolic acids. Apart from caffeic and chlorogenic acids, other phenolics are correlated with calcium. Gallic, rosmarinic and syringic acids are positively correlated with calcium attaining the correlation coefficients higher than 0.38 while ferulic acid is negatively correlated with this element (r = -0.37). Among 6 pairs of metals with caffeic acid, only two, zinc and sodium, were significantly correlated with this acid. In contrast to this, none of the studied metals correlated with chlorogenic acid. Moreover, no significant correlations were found between iron, magnesium and potassium and all the phenolics.

In the literature there is still a paucity of information on correlations between the essential metals and phenolic acids in herbs and spices. However, Ducat et al. [1] submits a hypothesis that correlations between aluminium, calcium and copper and total flavonoids in medicinal plants are caused by formation of flavonoid chelates with these ions. According to Maisuthisakul et al. [13], iron and calcium negatively correlates with total phenolic level expressed as gallic acid equivalent for 28 studied herbs and spices.

### Table 5: Statistically significant correlation coefficients for essential metals and phenolic acids.

|                | Zinc  | Sodium | Calcium | Chlorogenic acid | Gallic acid |
|----------------|-------|--------|---------|------------------|-------------|
| Iron           | 0.37  |        |         |                  |             |
| Sodium         | -0.42 |        | -0.45   |                  |             |
| Magnesium      | 0.37  |        |         |                  | 0.57        |
| Caffeic acid   | -0.36 | 0.38   |         | 0.37             |             |
| Ferulic acid   |        |        |         | -0.37            |             |
| Gallic acid    |        |        |         | 0.42             |             |
| Rosmarinic acid| 0.40  | 0.90   |         |                  | 0.60        |
| Syringic acid  | 0.38  |        |         |                  |             |

### Figure 3: Correlations between the levels of: (a) chlorogenic acid, (b) calcium, and rosmarinic acid in herbs and spices.
Thai plants. Furthermore, Sulaiman et al. [33] have shown that, with the exception of manganese, no correlation existed between macro- and trace elements and the total antioxidant capacity expressed by a radical scavenging DPPH assay of the banana fresh pulps and peels. Based on these reports, it is difficult to find an explanation for significant relationships among the studied phenolics and some metals (Table 5, Fig. 3b). Presumably the metals assist as cofactors of enzymes in biochemical transformations by which phenolics are synthetized. However, further research is needed to confirm this hypothesis.

4 Conclusions

In general, this study has shown that herbs are richer in essential metals and phenolic acids than spices. All plants contain high levels of calcium, potassium and rosmarinic acid but low levels of zinc and gallic acid. Taking into account the mineral content, lovage herbs and spices of thyme seemed to be the most abundant in metals. Correlation analysis revealed that significant correlations (p < 0.05) could be found between calcium and ferulic, gallic, rosmarinic and syringic acids as well as between zinc and sodium and caffeic acid. This suggests co-operation between these constituents in metabolic processes occurring in plants.

The multivariate analysis enabled identification of several clusters grouping plant samples (leaves of lemon balm, rosemary, mint and sage as well as caraway and lovage spices) originating from plants of a particular botanical species. It was also found that phenolic acids contents had a stronger impact on discrimination of the herbs and spices than the contents of metals. This study also confirmed the literature data that multivariate methods, PCA and HCA, can be successfully used to investigate correlations between the essential metals and phenolic acids in herbs and spices.

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