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Morphometrics and chemometrics as tools for medicinal and aromatic plants characterization

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Summary

The characterization of commercialized and consumed plants is of extreme importance, in order to provide clear data regarding the quality of plants, but also concerning the intake, by consumers, of several important compounds present is those plants. Hence, the objective of this work was to provide a detailed morphological and biochemical description of commercial samples of five common medicinal and aromatic plants (MAP's) (*Coriandrum sativum* L. –coriander, *Mentha spicata* L. – spearmint, *Ocimum basilicum* L. – basil, *Origanum vulgare* L. – oregano and *Petroselinum crispum* Mill. – parsley). For the studied species, statistically significant differences were evident for all the morpho-analytical characteristics investigated, as well as for the majority of the evaluated biochemical parameters. Specific leaf area was higher in *Coriandrum sativum*, while the water content of the leaves of *Ocimum basilicum* was the highest. Regarding photosynthetic pigments, when statistical differences were detected, these indicated the presence of larger amounts of chlorophyll and carotenoids in the leaves of *Coriandrum sativum* and *Petroselinum crispum*. Carbohydrate quantification indicated a considerably higher content in *Petroselinum crispum*, which also contained higher concentrations of protein, together with *Coriandrum sativum*. Quantification of total phenolic and thiobarbituric acid reactive substances indicated that they were correlated, with leaves of *Mentha spicata* presenting the highest values, on both parameters. This work provides an overview of selected characteristics of MAP's that are available for purchase, and are actually consumed by consumers.

Data are publicly available from the open access repository OpenAgrar, doi: 10.5073/openagrar.2017.000001.

Keywords
Coriandrum sativum L., *Mentha spicata* L., *Ocimum basilicum* L., *Origanum vulgare* L. and *Petroselinum crispum* Mill.; Biochemical characterization; Morphological traits

Introduction

Humans have always relied on the collection of natural resources for their needs, including several commodities, used for all purposes, from shelter to clothing or medicinal uses (SCHIPPMANN et al., 2006). For this latter intent, plants play a pivotal role, not only for their medicinal applications, but also for their food use and as trade goods (SCHIPPMANN et al., 2006). Indeed, from ancient Egypt, Rome and Greece to present days, plants and spices have been used as medicines and food preservatives (KAEFER and MILNER, 2008). All these characteristics, combined with the current scientific knowledge available on their properties, have increased the interest and demand for medicinal and aromatic plants (MAP's). The recommendation that wild species should be propagated and cultivated for commercial use (LAMBERT et al., 1997), was supported for two main reasons: from a sustainability point of view, since this would also serve conservation interests, and from a production point of view, since it would permit better control of biotic and abiotic production conditions) (SCHIPPMANN et al., 2006). Furthermore, cultivation of MAP's allows the possibility of biotechnological solutions for intrinsic problems associated with these types of plants, including species misidentification, genetic and phenotypic variability, variability and instability of extracts, toxic components and contaminants (YI and WETZSTEIN, 2010). Although negative features can also be attributed to the cultivation of MAP's, such as environmental degradation, loss of genetic diversity and loss of incentives to conserve wild populations (SCHIPPMANN et al., 2006), the consumer awareness of their benefits also boosted to the cultivation of MAP's, making them more readily available for purchase in commercial outlets. In fact, as for other plants, MAP's produce secondary plant metabolites, including phenolic compounds, which have crucial roles in human health. Some studies have pointed out the higher antioxidant activity of MAP's, when compared to fruits and vegetables (DRAGLAND et al., 2003). In fact, in recent years, a large number of studies have been devoted to the antioxidant activity of MAP's, as well as to their composition and morphological characteristics. However, most of those studies are on non-cultivated samples, as about two-thirds of the 50000 different medicinal plant species in use are collected from the wild (EDWARDS, 2004). In Europe, only 10 % of medicinal species used commercially are cultivated (VINES, 2004). Recent data indicates that the area of Portugal covered by MAP's production has grown six times in seven years (from 230 ha to 1324 ha, in 2004 and 2011, respectively), and, by 2012, a positive commercial balance was achieved, with exports surpassing imports of MAP's (GPP, 2012). Coriander (*Coriandrum sativum*), spearmint (*Mentha spicata*), basil (*Ocimum basilicum*), and parsley (*Petroselinum crispum*) are some of the MAP's that have more area devoted to their cultivation, to be commercialized as fresh plants, while oregano (*Origanum vulgare* L.) culture is mainly used for the production of dry product (GPP, 2012). Although a considerable volume of work is already available, concerning the characterization of these five MAP's, demonstrating the importance of, and interest in, these plant species, most studies have focused on non-cultivated specimens, rather than on their cultivated counterparts. This fact leaves an important part of the consumed MAP's fraction without available morphological and chemical data, useful to characterize actual plants used for human consumption. Hence, the objectives of this work are to morphometrically characterize the leaves of five MAP's (*Coriandrum sativum* L., *Mentha spicata* L., *Ocimum basilicum* L., *Petroselinum crispum* Mill. and *Origanum vulgare* L.), while also performing biochemical analysis of the referred plant species.

Materials and methods

Plant material

The following plants were purchased, potted, from a supermarket chain: *Coriandrum sativum*, *Mentha spicata*, *Ocimum basilicum*, *Origanum vulgare* and *Petroselinum crispum*. All plants were visu-

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ally inspected, and only completely healthy plants were selected. All the plants were from the same producer, were cultivated under similar conditions, and were purchased on the same day. The selection of potted and commercially available samples was made to evaluate the properties of plants actually used by consumers.

Biometric characterization

For the characterization of morphological parameters of the leaves of the studied plants, ten healthy and totally expanded mature leaves were selected, and the WinDIAS Leaf Area Meter System software (Delta-T Devices Ltd, Cambridge, United Kingdom) was used for the recording of data (area, length, width and perimeter). Moisture content (WC) was determined by oven-drying at 70 °C until constant mass was obtained. Shape factor is defined as the ratio of the actual perimeter (P) to that of a circle with the same area (P_c) (SF=P/Pc), while specific leaf area (SLA) is obtained using the relation leaf area/ dry mass. For histological analysis, leaf cross sections of healthy leaves were prepared for optical microscope examination (Olympus Mod. 1X 51; Olympus Optical Co., GmbH, Hamburg, Germany), using an Olympus Colorview III camera. Leaves were prepared by fixation in FAA (formalin-acetic acid-alcohol, 5:5:90 v/v), for 24 hours. Afterwards, cross sections were placed in 70% ethanol, and dehydration was achieved by immersing them 1 hour, in increasing ethanol concentrations (70%, 80%, 90%, 95% and 100%). Leaf samples were cleared by placing them in xylene, for 1 hour, after which it was embedded overnight in liquid paraffin, using a Leica EG1160 paraffin embedding station. The leaf material was cut using a Leica RM 2135 Rotary Microtome. De-paraffinization was performed using xylene, and hydration achieved by down-grading (100% - 70%) ethanol solutions. Staining with toluidine blue (0.1% for 7 minutes) preceded washing with water and new dehydration with ethanol. A last clearing step was performed with xylene, and mounting was completed in Entellan (Merck, Darmstadt, Germany).

Biochemical characterization

For the biochemical characterization of the studied plants, totally expanded mature leaves, from ten plants of each species were collected. From these leaves, ten 8 mm (diameter) discs were sampled. The discs were pooled, ground to a fine powder in a mortar with a pestle in the presence of liquid nitrogen and stored at -80 °C until further analysis. Biochemical characterization was performed in six sub-samples of the ground leaves.

Photosynthetic pigments

For the quantification of photosynthetic pigments, chlorophyll a (Cla), b (Clb) and total chlorophyll (Clt), and total carotenoids were spectrophotometrically determined from 80% acetone extracts, using the methods proposed by SESTÁK et al. (1971) and LICHTENTHALER (1987), respectively.

Soluble sugars and starch

The quantification of soluble sugars was performed using the method described by IRIGOYEN et al. (1992). Briefly, ground leaf was extracted with 10 ml of 80% ethanol, at 80 °C, for 1 hour. Afterwards, 0.2 mL of the alcoholic extract and 3 mL of anthrone was added and the mixture was placed in a water bath at 100 °C, for 10 minutes. Following extraction, the solid fractions obtained in the soluble sugar quantification was used for the starch analysis. Extraction was performed using 30% perchloric acid (OSAKI et al., 1991) and quantification followed the anthrone procedure described in the soluble sugars methodology, using also glucose as standard.

Soluble proteins

Total soluble proteins content was quantified as proposed by BRADFORD (1976). Leaf discs were ground in a buffer medium containing 50 mM phosphate buffer (pH 7.8), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 100 mM phenylmethylsulfonyl fluoride (PMSF) and 2% (w/v) polyvinylpyrollidone (PVP), and centrifuged at 22000 g for 30 minutes, at 4 °C. Absorbance was read at 595 nm, and bovine serum albumin (BSA) was used as a standard.

Total phenolics

The determination of total phenolics was performed in the same extracts used for the quantification of photosynthetic pigments. The methodology described by SINGLETON and ROSSI (1965) was followed, and quantification was achieved by spectrophotometric readings at 765 nm, using gallic acid as the reference standard, and expressed as mg gallic acid equivalents (GAE's).

Thiobarbituric acid reactive substances determination (TBARS)

The degree of lipid peroxidation was evaluated using the method described by BACELAR et al. (2006). Briefly, frozen leaf samples were ground with 2 mL of 20% (w/v) trichloroacetic acid (TCA) using a mortar and pestle. The mixture was centrifuged (3500 g; 20 minutes), and 1 mL of supernatant was combined with 1 mL 20% (w/v) TCA containing 0.5% (w/v) of thiobarbituric acid and 100 µL 4% (w/v) butylated hydroxytoluene (BHT). Thereafter, the mixture was heated at 95 °C for 30 minutes, cooled in an ice bath and again centrifuged at 10,000 × g for 10 minutes. The absorbance of the samples was obtained at 532 nm, whereafter the non-specific absorbance recorded at 600 nm was subtracted.

Statistical analysis

Data are presented as the mean ± standard deviation, expressed by fresh weight or leaf area, and differences among means were determined by analysis of variance (ANOVA), using SPSS (Statistical Package for Social Sciences) version 19.0 software (IBM Corporation, New York, U.S.A.). Averages were compared using the Duncan test ($P < 0.05$). Pearson correlations were calculated using SPSS. Principal component analysis (PCA) allows the recognition of patterns in the data by plotting them in a multidimensional space using the newly derived variables as dimensions (factor scores). PCA was applied for reducing the number of variables (30 variables corresponding to all analyzed parameters) to a smaller number of newly derived variables (principal component or factors) that adequately summarize the original information, i.e., the biometric and biochemical characterization of leaves of five MAP's. The factors to retain in data treatment were evaluated by the Scree plot, taking the eigenvalues, which should have be greater than one, into account, hence keeping the factor in the analysis and the internal consistency by means of αCronbach's value (MAROCO, 2003).

Results and discussion

Morpho-Anatomical Determinations

The leaves of all five MAP's presented a pinnate venation, consisting of double bundles, including the xylem and phloem, and upper and lower epidermal layers were clearly visible (Fig. 1). In all five plant species, the mesophyll tissues were differentiated into a single layer of palisade parenchyma towards the adaxial side, and spongy parenchyma on the abaxial side. It was also observed that the leaves of spearmint, oregano and parsley have a denser mesophyll (Fig. 1D, 1H and 1J, respectively) than coriander or basil leaves (Fig. 1B, Fig. 1F, respectively).

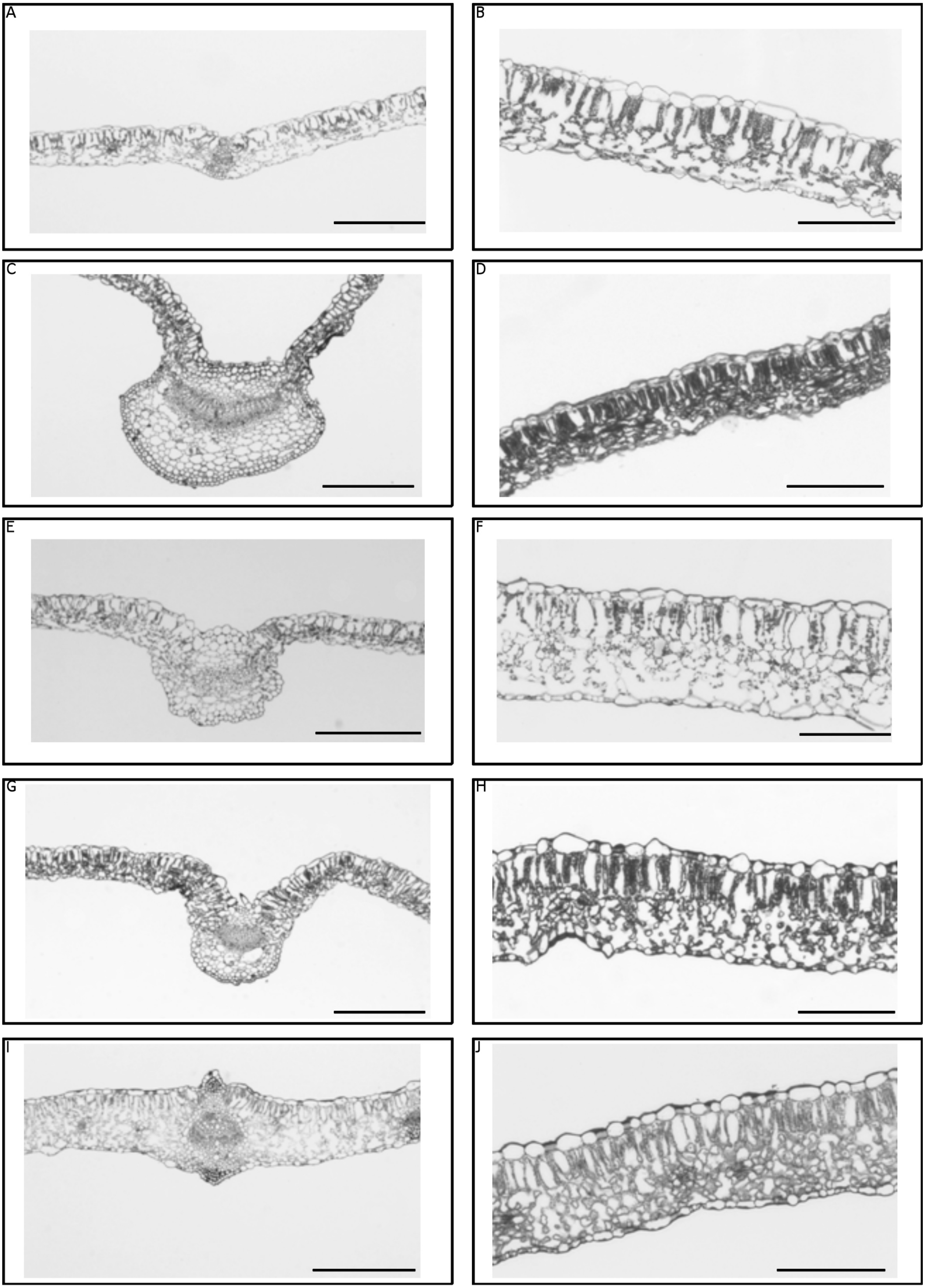

Fig. 1: Cross-sections of leaves of *Coriandrum sativum* (A and B), *Mentha spicata* (C and D), *Ocimum basilicum* (E and F), *Origanum vulgare* (G and H) and *Petroselinum crispum* (I and J). Bars on A, C, E, G and I of 500 µm and on B, D, F, H and J of 200 µm

Spearmint leaves presented on average a higher area (26.21±4.09 cm^2), length (8.66±0.52 cm) and width (4.38±0.40 cm) (Tab. 1). On the other hand, oregano was characterised by the lowest leaf area (4.31±0.69 cm^2), length and width (3.07±0.32 and 2.24±0.25 cm). For basil, the values for leaf area are similar to those described (Omidbaigi et al., 2010), while for oregano, our leaves were larger than those reported previously in the few available studies, which indicate areas of about 1.90 cm^2 (Panou-Filotheou et al., 2001) or between 1.01±0.17 to 3.71±0.49 cm^2 (Mastro et al., 2004). Regarding spearmint, parsley and coriander, no previous reports on this parameter were found. As for leaf area, studies regarding leaf dimensions (length and width) of the five studied MAP's are also few. Average values for length and width of leaves from nine Iranian spearmint landraces were reported to be 4.33 and 2.11 mm, respectively (Zeinali et al., 2004), with length ranging from 3.3 to 5.1 cm, and width ranging from 1.4 to 2.3 cm, lower than the measurements recorded in the present work (length of 8.66±0.52 cm and width of 4.38±0.40 cm). Only one previous study regarding basil leaf length and width is available (Nurzyñska-Wierdak, 2014). They reported a leaf length range from 34.6 to 70.2 mm, and width from 15.1 to 38.5 mm, which are comparable to our results. Regarding oregano, leaves with an average length of 26.7 mm (ranging from 10 to 50 mm) and an average width of 13.8 mm (ranging from 5 to 25 mm) were reported (Radušien and Stankevi, 2005). These values are similar to the results obtained in the present study (3.07±0.32 and 2.24±0.25 cm, respectively, for length and width). No data for length and width of parsley leaves were found in earlier works dealing with this plant. Measurement of leaf perimeter shows a significant effect of the plant species on this parameter. Results show higher values for parsley (36.93±12.52 cm), while a shorter perimeter was recorded for oregano (11.73±0.92 cm). Clear relations are visible between perimeter and other parameters, namely in spearmint, that presented larger area, length, width and diameter, and for oregano, that presented, for those parameters, the lowest values. Interestingly, parsley presented in-between values of area, length and width, but had the highest perimeter, due to the pronounced serrations on the leaves.

The results obtained for the leaf shape factor (Tab. 1) showed a higher value of this parameter for parsley (3.97±0.73), followed by coriander (3.34±0.77). On the other hand, lower values for shape factor were calculated for oregano (1.60±0.10), basil (1.79±0.28) and spearmint (2.02±0.09), although without statistically significant differences between them. Leaf shape factor, as defined here, indicates, for values closer to one, a rounder shape of the leaves. As expected, due to the shape of the leaf, these values are lower for oregano, basil and spearmint. In contrast, the leaf shape factor was higher for parsley and coriander, as these species present compound leaves, in some cases with strong serration, thereby increasing the measured perimeter.

Specific leaf area (SLA) (m^2/kg) is often positively related to potential relative growth rate, but also to leaf density, as well as to leaf thickness, although the influence of each of these factors depends on habitat and plant group (Pérez-Harguindeguy et al., 2013). Our results showed significant influence of the species in this parameter ($P = 0.0001$) (Tab. 1). Higher SLA was found in leaves of coriander (47.97±0.96 m^2/kg), while parsley presented the lower values (23.55±0.71 m^2/kg). Previous work, regarding basil, present values of SLA very similar to the data recorded in the present work (35.18 m^2/kg) (Chang et al., 2008). For spearmint, similar SLA was found in field-grown plants (23.1±3 m^2/kg), while significantly lower values were observed in greenhouse plants (15.1±2 m^2/kg) (Yi and Wetzstein, 2010). No reports were available regarding SLA in the remaining studied plants. Water content (%) (Tab. 1) was heavily dependent on studied plant species, varying from 81.33±0.98% in spearmint, to 91.68±0.66% in basil. The recorded values of water content for leaves of spearmint are noticeably higher than those previously reported by Yi and Wetzstein (2010), either in greenhouse (70±9%) or field plants (85±3%). It was concluded that the higher moisture content was related to the growing environment. The moisture content of coriander was found to be similar to the 87% reported by Divya et al. (2012).

The leaf morpho-anatomical characteristics, besides influencing plant growth and biochemical parameters, are also likely to influence consumer preference, quality and post-harvest storage period, although few works are devoted to this latter topic. For instance, and regarding the influence of leaf characteristics and plant growth and biochemical parameters, a comprehensive study, performed by Poorter and Remkes (1990), highlighted, for 24 plant species, a clear relationship between growth rate and leaf morpho-anatomical characteristics, namely specific leaf area, the ratio between leaf area and leaf weight, and leaf weight ratio. A similar result for SLA was reported by Chaturvedi et al. (2011). Furthermore, leaf area plays a key role in photosynthesis, light interception, water and nutrient use, crop growth and development (Caliskan et al., 2010). The effects that leaf traits, specifically morpho-anatomical ones, have on the post-harvest storage period have been studied, usually with regard to leaf drying-procedures or minimally processed fresh-cut vegetables. In these studies, water content is an important factor, considering the drying of medicinal and aromatic plants (Müller and Heindl, 2006; Soysal and Öztekin, 1999), as it is leaf area, that determines the surface area available for water loss, favouring quick drying (Tanko et al., 2005). In contrast, no previous data could be found regarding how quickly recognized leaf traits, such as leaf size and shape, affect consumer choice when purchasing a MAP, or how those traits affect the sensorial characteristics of leaves.

Biochemical determinations

The quantitative results of photosynthetic pigments determined in the plants are presented in Tab. 2. Regarding Cla content per leaf

Tab. 1: Morpho-anatomical parameters (area, length, width, shape factor, perimeter), specific leaf area (SLA) and water content of leaves of the five studied species of MAP's (mean ± SD, n = 10).

Plant	Area (cm^2)	Length (cm)	Width (cm)	Perimeter (cm)	Shape factor	SLA (m^2/kg)	Water content (%)
***Coriandrum sativum* L.**	6.01±1.78 ab	4.11±1.03 b	2.94±0.58 b	29.41±9.70 c	3.34±0.77 b	47.97±0.96 d	87.94±1.25 c
***Mentha spicata* L.**	26.21±4.09 d	8.66±0.52 d	4.38±0.40 c	36.41±2.28 d	2.02±0.09 a	23.63±0.54 a	81.33±0.98 a
***Ocimum basilicum* L.**	10.58±1.59 c	5.44±0.44 c	2.99±0.58 b	20.48±3.15 b	1.79±0.28 a	36.80±0.47 c	91.68±0.66 d
***Origanum vulgare* L.**	4.31±0.69 a	3.07±0.32 a	2.24±0.25 a	11.73±0.92 a	1.60±0.10 a	26.63±0.46 b	83.29±1.85 b
***Petroselinum crispum* Mill.**	7.16±3.61 b	4.53±1.42 b	3.30±0.86 b	36.93±12.52 d	3.97±0.73 c	23.55±0.71 a	81.39±1.21 a
P	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*

Means within a column followed by the same letter are not significantly different at $P < 0.05$, according to the Duncan multiple range test.

Tab. 2: Photosynthetic pigments (mg/g or mg/dm^2 of fresh weight) content of leaves of the five studied species of medicinal and aromatic plants (mean ± SD, n = 6).

Plant	Chlorophyll a (mg/dm^2)	Chlorophyll a (mg/g)	Chlorophyll b (mg/dm^2)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/dm^2)	Total Chlorophyll (mg/g)	Chlorophyll a/ Chlorophyll b	Total Carotenoid (mg/ dm^2)	Total Carotenoid (mg/g)	Total Chlorophyll Total Carotenoid
***Coriandrum sativum* L.**	2.63±0.38 ab	13.14±1.90 d	1.11±0.66 a	5.56±3.27 b	3.74±0.99 a	18.69±4.88 c	2.72±0.74 bc	0.59±0.08 ab	2.92±0.39 d	6.58±2.57 a
***Mentha spicata* L.**	2.32±0.43 a	5.48±1.02 a	1.67±0.72 a	3.95±1.71 ab	3.99±1.15 a	9.43±2.71 a	1.61±0.67 a	0.52±0.06 a	1.22±0.15 a	7.79±2.37 a
***Ocimum basilicum* L.**	2.67±0.25 abc	9.84±0.92 c	1.14±0.53 a	4.22±1.97 ab	3.82±0.76 a	14.06±2.36 b	2.58±0.66 bc	0.60±0.04 b	2.23±0.14 c	6.36±1.52 a
***Origanum vulgare* L.**	3.07±0.25 c	8.14±0.64 b	1.05±0.21 a	2.79±0.58 a	4.12±0.43 a	10.93±1.16 ab	2.98±0.42 c	0.72±0.06 c	1.92±0.17 b	5.72±0.73 a
***Petroselinum crispum* Mill.**	2.79±0.39 bc	6.64±0.88 a	1.55±0.66 a	3.67±1.52 ab	4.34±1.03 a	10.30±2.36 a	2.03±0.67 ab	0.73±0.07 c	1.75±0.16 b	5.93±1.34 a
P	*0.2040*	*0.0001*	*0.2656*	*0.2315*	*0.7994*	*0.0001*	*0.0072*	*0.0001*	*0.0001*	*0.3462*

Means within a column followed by the same letter are not significantly different at $P < 0.05$, according to the Duncan multiple range test.

area (mg/dm^2), no significant effect of the plant species was detected ($P = 0.2040$), with values varied between 2.32±0.43 mg/dm^2, in spearmint, to 3.07±0.25 mg/dm^2 in oregano. When Cla was quantified by leaf weight (mg/g), the plant species played a determining role ($P = 0.0001$), with higher values recorded for coriander (13.14±1.90 mg/g), while spearmint presented the lowest values of Cla (5.48±1.02 mg/g). The values for Cla quantification achieved in the present work are, in most cases, higher than previously reported, for all the studied plants (Bernstein et al., 2010; Corrêa et al., 2009; Costa et al., 2013; Keser and Buyuk, 2012; Singh et al., 1999; Verma and Sen, 2008), while a similar value was obtained by Murillo-Amador et al. (2013) for oregano leaves.

The specific plant species had no influence on the concentrations of Clb, per leaf area ($P = 0.2656$). As for Cla, the content of Clb was considerably higher than previous reports indicate. Indeed, all available reports for the studied plants provide lower values for this photosynthetic pigment (Bernstein et al., 2010; Corrêa et al., 2009; Costa et al., 2013; Divya et al., 2012; Keser and Buyuk, 2012; Murillo-Amador et al., 2013; Singh et al., 1999; Stancheva et al., 2014).

Total chlorophyll concentration (Tab. 2) did not differ significantly among species ($P = 0.7994$), as expressed by leaf area. However, when results are expressed per gram of leaf, significant differences in the Clt content became evident between the species ($P = 0.0001$). Coriander contained the most Clt per gram of leaf (18.69±4.88 mg/g), which was considerably higher than the amount determined in spearmint (9.43±2.71 mg/g). Again, our results indicated higher levels of Clt than those previously reported for all analyzed plants (Corrêa et al., 2009; Costa et al., 2013; Panou-Filotheou et al., 2001; Singh et al., 1999; Verma and Sen, 2008; Baranauskienė et al., 2013; Bekiaroglou and Karataglis, 2002; Lers et al., 1998; Najla et al., 2012; Sakalauskaitė et al., 2012). Only one group (Asrar et al., 2005) reported higher concentrations (12 to 25 mg/g fresh weight) of Clt in spearmint leaves, when grown under excess manganese, than the values recorded in the present study. The ratio Cla/Clb in plants is normally approximately 3, but lower in shade leaves (Clydesdale and Francis, 1968). In the current study, oregano (2.98±0.42), coriander and basil (2.72±0.74 and 2.58±0.66, respectively) presented values close to 3. Lower ratios were determined in both parsley (2.03±0.67) and spearmint (1.61±0.67). Previous reports have shown different Cla/Clb ratio, in relation to our values. For oregano, ratio of over 3.5 and, for basil, ratio around 3.3 was reported (Baranauskienė et al., 2013). Also, lower ratios were found in oregano, reaching values of 1.4 (Stancheva et al., 2014), or 1.72 and 1.78 (Baranauskienė et al., 2013). Low Cla/Clb ratios, simultaneously with low amounts of Clt, has detected for spearmint, may indicate that there is an increase of the light-harvesting complex chlorophyll a/b-protein, relatively to the content of total chlorophyll of the chloroplasts (Candolfi-Vasconcelos, 1990).

Carotenoids have two well-known functions in photosynthetic processes: they serve as accessory pigments in light harvesting and as photoprotectors against oxidative damage, due to their ability to quench singlet oxygen, minimizing also its formation, and by absorbing excess energy from excited triplet states of chlorophyll (Siefermann-Harms, 1987). The content of carotenoids (Tab. 2) in the studied plants was significantly influenced by the species ($P = 0.0001$), and varied from 0.52±0.06 mg/dm^2 in spearmint to 0.73±0.07 mg/dm^2 in parsley. When the results were expressed in mg/g, spearmint contained the least (1.22±0.15 mg/g), while the highest concentration was present in coriander leaves (2.92±0.39 mg/g). Following the same trend as for the chlorophylls, the content of carotenoids determined was in almost all cases higher than reported values (Bernstein et al., 2010; Capecka et al., 2005; Costa et al., 2013; Keser and Buyuk, 2012; Sakalauskaitė et al., 2012).

Total chlorophyll to total carotenoids ratio is regarded as a plant stress response (Hendry and Price, 1993). This ratio tends to be reduced in leaves exposed to conditions of low light (Levizou and Manetas, 2007). In the present work, total chlorophyll/total carotenoids values did not show significant differences between plant species ($P = 0.3462$). Only a few researchers have reported on this specific parameter. Stancheva et al. (2014) indicated a much lower chlorophyll/carotenoids ratio for basil (2.75, comparing to 6.36±1.52), but a similar ratio for oregano (5.33, comparing to 5.72±0.73).

The concentrations of soluble sugars determined in each of the species are presented in Tab. 3. Concentration of soluble sugars has been correlated with specific leaf mass (kg/m^2) (Castrillo et al., 2005) and to irradiance, where higher carbon acquisition is possible, due to increased photosynthesis (Niinemets, 1997). It is also known that water stress induces the accumulation of soluble sugars in leaves (Quick et al., 1992), because these are able to act both as osmoprotectants and carbon sources (Chaves et al., 2002). The results obtained revealed significant differences between plant species ($P = 0.0001$), and ranged from 21.38±4.89 mg/dm^2 in coriander to

Tab. 3: Soluble sugars, starch and non-structural carbohydrates content (mg/g or mg/dm^2 of fresh weight) of leaves of the five studied species of medicinal and aromatic plants (mean ± SD, n = 6).

Plant	**Soluble sugars (mg/dm^2)**	**Soluble sugars (mg/g)**	**Starch (mg/dm^2)**	**Starch (mg/g)**	**Soluble sugars/ Starch**	**Non-structural carbohydrates (mg/dm^2)**	**Non-structural carbohydrates (mg/g)**
***Coriandrum sativum* L.**	21.38±4.89 a	102.66±24.15 a	13.34±3.14 a	63.88±14.40 a	1.74±0.83 a	34.73±3.81 a	166.54±17.91 a
***Mentha spicata* L.**	61.99±5.86 c	146.28±11.30 b	34.48±5.66 c	81.49±13.71 ab	1.85±0.38 a	96.48±4.49 c	227.77±7.88 b
***Ocimum basilicum* L.**	22.96±6.25 a	84.33±22.14 a	22.77±9.40 b	84.11±35.52 ab	1.25±0.76 a	45.74±5.50 a	168.44±21.34 a
***Origanum vulgare* L.**	36.99±4.88 b	98.51±12.86 a	35.55±5.70 c	94.55±14.44 b	1.08±0.29 a	72.53±5.79 b	193.07±13.53 a
***Petroselinum crispum* Mill.**	163.45±22.26 d	384.79±53.81 c	37.07±4.79 c	87.36±12.05 ab	4.48±0.89 b	200.52±21.69 d	472.15±53.06 c
P	*0.0001*	*0.0001*	*0.0001*	*0.1356*	*0.0001*	*0.0001*	*0.0001*

Means within a column followed by the same letter are not significantly different at $P < 0.05$, according to the Duncan multiple range test.

163.45±22.26 mg/dm^2 in parsley. When expressed per gram of leaf, the lowest content was obtained for basil (84.33±22.14 mg/g), being the highest amount of soluble sugars again quantified in parsley (384.79±53.81 mg/g). Few reports were devoted to the quantification of these compounds in the studied plants. For basil, similar values of soluble sugars were obtained by CASTRILLO et al. (2005) (2 g/m^2), while considerably lower values of soluble sugars were determined by COSTA et al. (2013) (around 1 g/kg fresh weight) and by KHOMDRAM et al. (2011) (2.55±0.100 mg/g dry weight).

In many plants, starch is one of the major products of photosynthesis, along with sucrose (BÜCHI et al., 1998), serving as short-term storage carbohydrates that can be accumulated during the day and remobilized at night to ensure the continuous availability of energy to the entire plant (ZEEMAN et al., 2007). These are also the main digestible carbohydrates in the human diet, and a major source of glucose (TIWARI et al., 2013). Variations of starch content between different plant species can be explained by the fact some species use starch as the major storage form of photoassimilates, while others accumulate both starch and sucrose (ZEEMAN et al., 2007). The starch content (Tab. 3), showed differences when quantification was done per leaf area or per mass. When the starch content was expressed as mg/dm^2, a significant effect of the plant species was detected ($P = 0.0001$). Indeed, coriander presented the lowest amount of starch (13.34±3.14 mg/dm^2), and the results were statistically different from those recorded for the other studied plants. Parsley (37.07±4.79 mg/dm^2) contained the highest content of starch. When the results were expressed in mg/g, there were no significant differences between the plant species ($P = 0.1356$). The literature is almost devoid of information related to the starch content of any of the plants studied. The only available report (BÜCHI et al., 1998) regarding basil, indicated considerable variation in the starch content (from around 1 mg/g to almost 70 mg/g fresh weight), depending on the plant age.

Soluble sugars to starch ratio was significantly influenced by the plant species ($P = 0.0001$). Results yielded similar values of this ratio for oregano (1.08±0.29), basil (1.25±0.76), coriander (1.74±0.83) and spearmint (1.85±0.38), while parsley presented a significantly higher average value (4.48±0.89). The ratio for parsley was affected by the high amounts of soluble sugars, which were at least four times higher than the lower content detected of these compounds, in the other studied plants. These variations of soluble sugars/starch ratio may be explained, as mentioned before, by different accumulation patterns for photo-assimilates by different species (HUBER, 1989). Our results suggest that for parsley, starch may be a less important storage carbohydrate. It seems that for the other plants, storage and other sugars are equally utilised, although further studies are needed for confirmation. Indeed, starch can be used transiently to store synthesised carbohydrates, while soluble sugars might serve as osmolytes during water stress (PALLARDY, 2008).

Results for non-structural carbohydrates reflect the data recorded for soluble sugars and starch quantification. As for the other parameters, variations in the content of non-structural carbohydrates is related to the specific biology of the plant species, and is also dependent on photosynthesis, respiration and growth (LI et al., 2013). Significant differences ($P = 0.0001$) were observed among plant species, either when results are expressed by leaf area or mass. Coriander presented the lowest value (34.73±3.81 mg/dm^2 and 166.54±17.91 mg/g) for non-structural carbohydrates, resulting from a low content of both soluble sugars (mg/dm^2) and starch. On the other hand, the higher amount of soluble sugars and starch (mg/g) present in parsley leaves are responsible for the highest amount of non-structural carbohydrates measured (200.52±21.69 mg/dm^2 and 472.15±53.06 mg/g).

Soluble protein content (Fig. 2) was influenced by the plant species ($P = 0.0001$), and varied greatly, from the minimum of 24.94±7.79 mg/dm^2 and 0.78±0.18 mg/g, detected for oregano, to the maximum of 545.08±52.02 mg/dm^2 in parsley, and 19.09±1.51 mg/g, in coriander. Few studies report on the soluble protein content of the presently studied plants. Only one report was found for spearmint and parsley, indicating a protein concentration of 2.3g/100g of fresh weight (SCHERER et al., 2013) and 15 mg/g of fresh weight (LERS et al., 1998), respectively.

The TBARS assay is one of the most widely used tests for the determination of lipid peroxidation, where the lipids to be analyzed

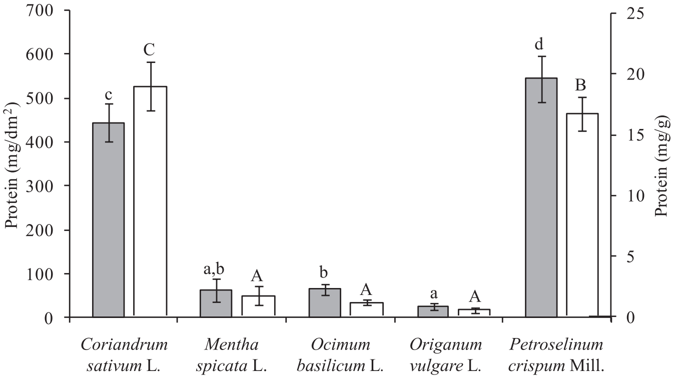

Fig. 2: Protein content of leaves of the five studied species of medicinal and aromatic plants (mean ± SD, n = 6). Grey bars with different letters indicate values with significant differences at $P < 0.05$ for content expressed as mg/dm^2, and white bars with different capital letters indicate values with significant differences at $P < 0.05$ for content expressed as mg/g of fresh weight.

Tab. 4: Total thiobarbituric acid reactive substances concentration (TBARS) and total phenolic content (mg/g or mg/dm^2 of fresh weight) of leaves of the five studied species of medicinal and aromatic plants (mean ± SD, n = 6).

Plant	**TBARS (nmol/dm^2)**	**TBARS (nmol/g)**	**Total phenolics (mg/dm^2)**	**Total phenolics (mg/g)**
***Coriandrum sativum* L.**	0.25±0.03 a	124.43±16.78 b	17.43±1.72 a	87.09±9.13 a
***Mentha spicata* L.**	0.94±0.13 c	221.45±33.32 d	103.33±12.87 c	243.78±28.82 c
***Ocimum basilicum* L.**	0.24±0.09 a	88.55±35.95 a	20.45±1.30 a	75.29±4.09 a
***Origanum vulgare* L.**	0.34±0.06 a	88.86±17.57 a	59.34±9.63 b	157.20±24.56 b
***Petroselinum crispum* Mill.**	0.66±0.06 b	156.96±15.65 c	65.57±3.15 b	156.12±5.55 b
P	*0.0001*	*0.0001*	*0.0001*	*0.0001*

Means within a column followed by the same letter are not significantly different at $P< 0.05$, according to the Duncan multiple range test.

are mixed with thiobarbituric acid (TBA). In our results (Tab. 4), a significant effect of the plant species was detected ($P = 0.0001$). Lower values of lipid peroxidation were found for basil (0.24±0.09 nmol/dm^2 and 88.55±35.95 nmol/g), while higher values of lipid peroxidation were determined for spearmint (0.94±0.13 nmol/dm^2 and 221.45±33.32 nmol/g). Only a few studies evaluated TBARS in the leaves of the investigated plant species. Lower values of TBARS (about 120 nmol/g dry weight) were determined by TARCHOUNE et al. (2010) and by RAMÍREZ-SANDOVAL et al. (2011) (about 15 nmol/g leaf), in basil leaves. It is known that lipid peroxidation is a natural metabolic process, and one of the most studied reactive oxygen species (ROS) actions on membrane structure and function (BLOKHINA et al., 2013). One of the mechanisms mainly used by plant to inactive ROS is the use of antioxidants, which react with the radical electron, but are stable in its presence, as a result of the presence of conjugated bonds (VERMERRIS and NICHOLSON, 2006). Phenolic compounds are highly effective antioxidants, and our results show a clear relation between the amount of TBARS and total phenolics (Tab. 3). Indeed, higher values of total phenolic content were found in the leaves of spearmint (103.33±12.87 mg gallic acid equivalents (GAE)/dm^2 and 243.78±28.82 mg GAE/g), that yielded higher values for TBARS. Furthermore, the lowest amount of total phenolic content, per gram of leaf (75.29±4.09 mg GAE /g) was measured in basil, which presented the lowest TBARS. The total phenolic content reported (0.94 mg (GAE)/g fresh weight) for spearmint leaves is very low when compared to our results (ZHENG and WANG, 2001). The total phenolic content of coriander yielded the lowest values, expressed in leaf area (17.43±1.72 mg GAE /dm^2) and per leaf mass (87.09±9.13 mg GAE /g), but still, considerably higher to those previously reported. In some studies the total phenolic content was as low as 7 µg/g of extract (NAIR et al., 2012), 49.2±3.34 mmol GAE/kg dry weight (KAISER et al., 2013) to 3.74 mg GAE/g fresh weight (ISABELLE et al., 2010) and 25.23±2.17 GAE/100g (SREELATHA and INBAVALLI, 2012). Basil showed a content of total phenolics of 20.45±1.30 mg GAE/dm^2 and 75.29±4.09 mg GAE/g, the lowest when expressed by leaf mass. The available reports concerning this plant and its phenolic content are more consistent, even though lower than the presently quantified. Hence, total phenolic content has been reported to be as low as 1.4 mg GAE/g fresh weight (SAKALAUSKAITĖ et al., 2012) similar to the values reported by ZHENG and WANG (2001) (2.23±0.15 mg of GAE/g of fresh weight) or DOĞAN et al. (2005) (280 mg/100g fresh weight). Oregano presented an intermediate content of total phenolics (59.34±9.63 mg GAE/dm^2 and 157.20±24.56 mg GAE/g), when compared to the other species. Several reports are available, regarding the phenolic content of oregano leaves. Low amounts of these compounds were reported by ZHENG and WANG (2001) (11.80±0.60 mg of GAE/g of fresh weight), 1406 mg GAE/100 g fresh weight (CAPECKA et al., 2005), while other researchers reported higher amounts of total phenolics (CHUN et al., 2005; MASLENNIKOV et al., 2014; SPIRIDON et al., 2011). Similar results to the ones obtained in the present study were also reported by ŠKERGET et al. (2005) (186 g GAE/kg extract). Parsley, which also presented an intermediate amount of total phenolics, when compared to the other studied plants (65.57±3.15 mg GAE/dm^2 and 156.12±5.55 mg GAE/g), has also been studied by other researchers, regarding total phenolics content. Those reports indicate total phenolic content that is significantly lower than our values, ranging from 1.12±0.01 mg of GAE/g of fresh weight (ZHENG and WANG, 2001) to 18.25±1.21 mg gallic acid/g dry weight (DORMAN et al., 2011) or 196.8 mg GAE per 100 g dry weight (RABABAH et al., 2011), or even higher (27 mg gallic acid equivalent/g fresh weight, SEZGIN et al., 2010). Several health-promoting effects of the studied plants have been reported, including antioxidant, anticancer and antimicrobial activity, as well as hypoglycaemic and anti-diabetic properties. This is a clear indication that these species may be beneficial in reducing cardiovascular disorders (CRAIG, 1999; GURIB-FAKIM, 2006) with those effects linked mainly to the content and composition in phenolics compounds, but also related to carbohydrate and carotenoid content. As consumers do not weigh the amount of leaves that they are consuming, but instead visually control how much they are using, one should consider the results expressed per leaf area to indicate the most interesting plant. Hence, parsley and spearmint appear to be more prone to have enhanced beneficial effects in human health, due to their content of those helth-promoting compounds.

Biochemical parameters have also been studied, in MAP's, regarding post-harvest conditions and optimization procedures for storage. However, the large majority of studies reflect how the chemical composition is affected by those procedures, while the inverse should also be investigated. There are many studies focused on the the effect of the drying procedure, used to increase the storage, or the storage period itself, on several chemical properties of MAP's, including phenolic content (CAPECKA et al., 2005; LOUGHRIN and KASPERBAUER, 2011), ascorbic acid (AKBUDAK and AKBUDAK, 2013; SINGH et al., 1999), carotenoids (DELGADO-VARGAS et al., 2000; DIVYA et al., 2012), chlorophylls (CESARE et al., 2003; SILVA et al., 2005), as well as the antioxidant activity of these plants (GIÃO et al., 2013). Although no data were found on how chemical composition will affect drying and storage, it can be expected that leaves containing larger amounts of phenolics and carotenoids will be less prone to oxidation, as those compounds are proven antioxidants (KRINSKY, 1989; RICE-EVANS et al., 1997). Still regarding phenolics, their content can also influence leaf sensorial attributes, as they are associated with two main descriptors, i.e. bitterness and astringency, which are linked to negative consumer reactions (LESSCHAEVE et al., 2005). Similarly, leaves with high chlorophyll content will be less subject to colour changes from green to brown caused by drying, due to the degradation of chlorophyll to pheophytins (Cesare et al., 2003). This will subsequently influence consumer choice, which is likely to be influenced by the visual characteristics of the plants. Protein content has also been reported to reduce significantly with storage period (Singh et al., 2003). The soluble sugar content may vary with storage time in several MAP's (Büchi et al., 1998; Santos et al., 2014) and is linked to starch content and water content (Mohamed et al., 2005), which can ultimately influence sensorial characteristics (Barrett et al., 2010) and therefore consumer preference.

The use of morphological data for the construction of a principal component analysis (PCA) model (Fig. 3) allowed 83 % of the observed variance to be explained. As expected, a clear separation of the analyzed species is visible, as morphological data are significantly different among those plants. However, when using the significantly different data from biochemical analysis, the corresponding PCA model explained 69 % of the total variance (Fig. 4). A clear separation of plant species was observed, using this data. Coriander samples are all represented in the positive region of Factor 1 and 2, due to their high content of Cla (mg/g), Clt (mg/g) and total carotenoids (mg/g). Spearmint samples are located in the negative regions of both factors, due to the higher content of phenolic compounds, while parsley samples appear in the negative region of Factor 1 and positive region of Factor 2, due to its higher content on soluble sugars, and non-structural carbohydrates.

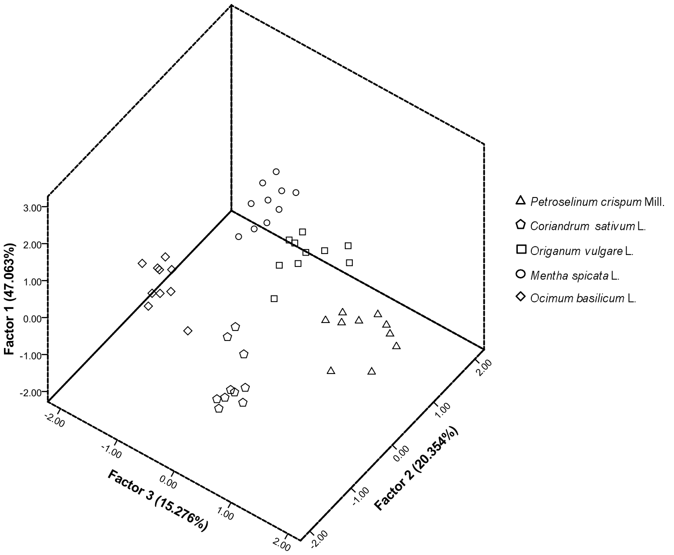

Fig. 3: Principal component analysis obtained from the morphological parameters of five different aromatic and medicinal plants. PCA factors explain 82.692 % of the total variance.

A principal component analysis (PCA) was also performed with all the data obtained, from both morphological, as well as biochemical analysis. In the present study, the total variance explained by the PCA was 57 % (Fig. 5), by using two principal components, allowing a good discrimination of all five plant species. In the positive region of Factor 1 and Factor 2 are represented the samples of coriander, mainly due to the higher values of Clb and Clt (mg/g), as well as total carotenoids (mg/g). On the other hand, the soluble sugar and carbohydrate content influenced the representation of parsley samples in the negative region of Factor 1 and positive region of Factor 2. Spearmint samples appear in the negative region of Factor 1 and Factor 2, due to higher area and length, and total phenolic content. The discrimination of basil and oregano samples, in the negative region of Factor 2 and positive region of Factor 1 resulted from the content on total carotenoids, as well as of Cla (mg/dm^2).

Several correlations between the analyzed parameters were found, although different patterns were exhibited by each plant species (Oliveira, 2017). Interestingly, only Cla, Clb and Clt content were found to be correlated in all studied plant species, together with the expected relation between area and width. Other correlations that must be mentioned occurred between the chlorophyll content (Cla, Clb and Clt, either in mg/g or mg/dm^2) and non-structural carbohy-

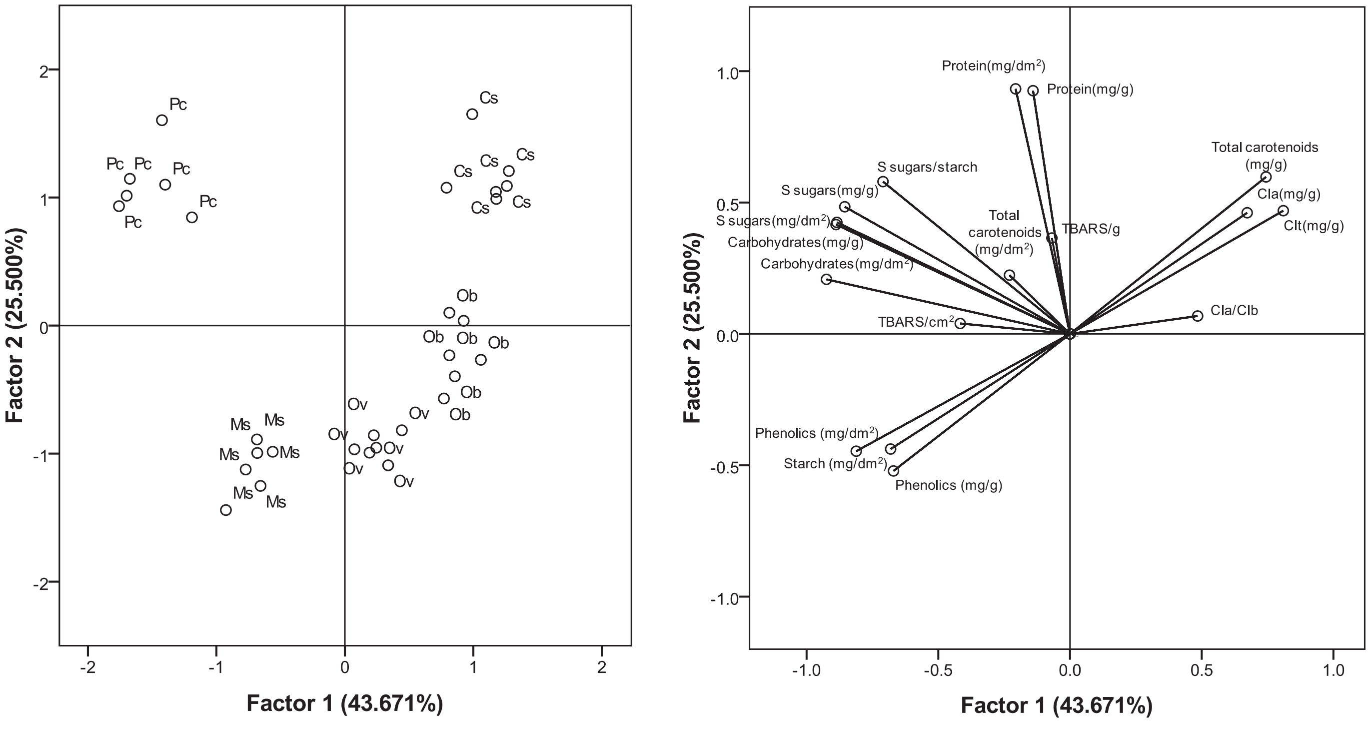

Fig. 4: Principal component analysis obtained from the significant different data of biochemical parameters of five different aromatic and medicinal plants (Cs – *Coriandrum sativum* L.; Ms – *Mentha spicata* L.; Ob – *Ocimum basilicum* L.; Ov – *Origanum vulgare* L.; Pc – *Petroselinum crispum* Mill.). PCA factors explain 82.692 % of the total variance.

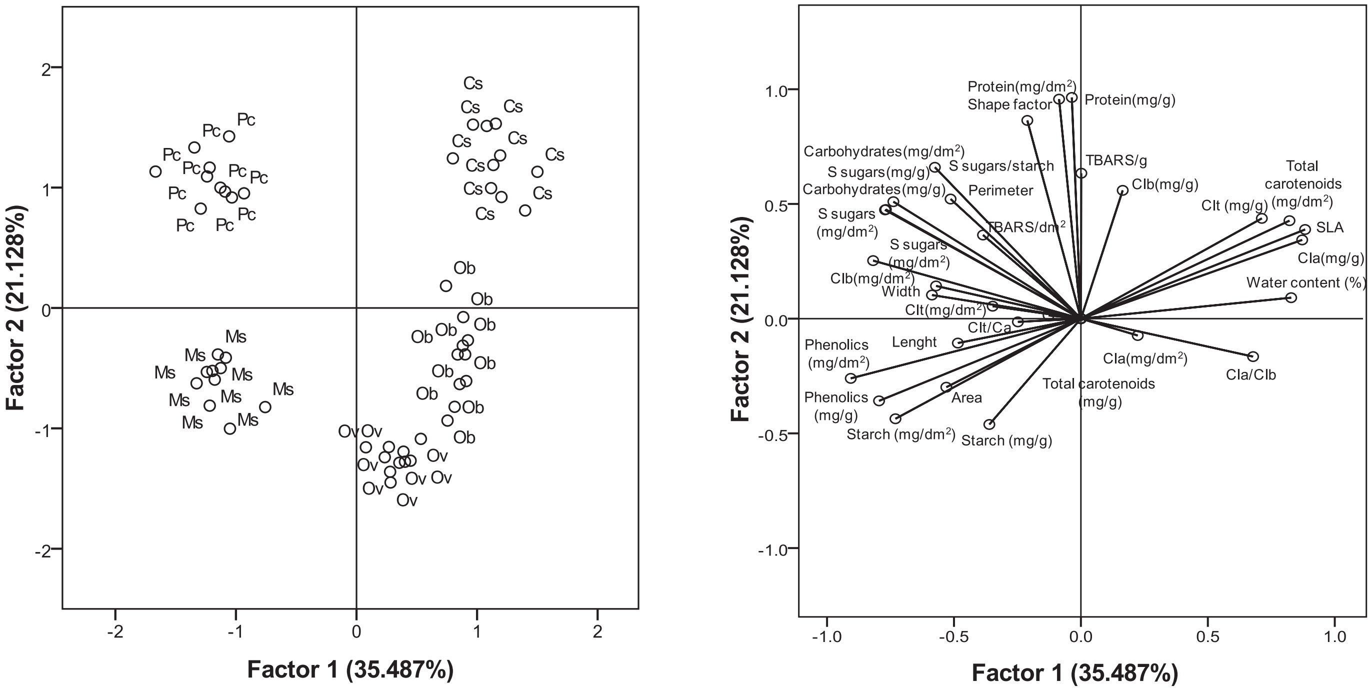

Fig. 5: Principal component analysis obtained from all analyzed parameters of five different aromatic and medicinal plants (Cs – *Coriandrum sativum* L.; Ms – *Mentha spicata* L.; Ob – *Ocimum basilicum* L.; Ov – *Origanum vulgare* L. Pc – *Petroselinum crispum* Mill.). PCA factors explain 56.615% of the total variance.

drates (also either in mg/g or mg/dm^2), only detected for oregano, where extremely significant (P=0.01) and positive correlations were found (Oliveira, 2017, tab *Oreganum*). This correlation between chlorophylls and non-structural carbohydrates is probably due to the higher photosynthetic production, caused by increased carbon acquisition (Niinemets, 1997). Several other correlations were only observed in one of the studied plants. Indeed, carotenoid and protein content (both either as mg/g or mg/dm^2) were only found to be nega-tively correlated in leaves of basil (Oliveira, 2017, tab *Ocimum*), while proteins where only negatively correlated to non-structural carbohydrates (both either as mg/g or mg/dm^2) in leaves of pars-ley (Oliveira, 2017, tab *Petroselinum*). In contrast, soluble sugars were found to be correlated to leaf area, but on only for coriander (Oliveira, 2017, tab *Coriandrum*), where a negative correlation was established, being also negatively correlated to carotenoid content in leaves of spearmint (Oliveira, 2017, tab *Mentha*) (all either as mg/g or mg/dm^2). Another correlation found in the leaves of only one of the studied plant species was the positive relation between protein and starch (both either as mg/g or mg/dm^2), detected in the leaves of coriander (Oliveira, 2017, tab *Coriandrum*). Other noteworthy correlations were the ones found with TBARS. Indeed, this parameter (either as mg/g or mg/dm^2) was positively correlated to the carotenoid content (mg/dm^2) in the leaves of oregano (Oliveira, 2017, tab *Origanum*), negatively correlated to Cla content (either as mg/g or mg/dm^2), in leaves of coriander and parsley (Oliveira, 2017, tabs *Coriandrum* and *Petroselinum*), as well as to Cla/Clb, Clb and Clt content (either as mg/g or mg/dm^2), for parsley leaves (Oliveira, 2017, tab *Petroselinum*). Positive correlation between TBARS and chloroplastic pigments (carotenoids and chlorophylls) have been re-ported in Fe-starved plants suggesting that, in those circumstances, decreased concentrations of chloroplastic pigments may contribute to protect plants from oxidative damages (Tewari et al., 2005). How-ever, a negative correlation could be expected, as chloroplastic pig-ments can act as inhibitors of oxidation processes and reduce the val-ues of TBARS (He and Shahidi, 1997). Finally, another correlation, in this case a positive one, only detected in the leaves of one plant, spearmint, was found between total phenolic content (either as mg/g or mg/dm^2) and chlorophyll (Cla, Clb and Clt) content (also either as mg/g or mg/dm^2) (Oliveira, 2017, tab *Mentha*). Some of the cor-relations found in the present study have already been reported in previous works. Good correlations between leaf area and water con-tent (Xu et al., 2009) and starch (Manas et al., 2014) have previously been reported, although in other plant species, as well as correlations to some phenolic compounds (Çirak et al., 2007). Furthermore, wa-ter content and other parameters have been reported to be correlated, namely to soluble sugars (Wenjiang et al., 2004), SLA (Li et al., 2010) and chlorophyll (Cañas et al., 1997). SLA has been correlated to carotenoids and proteins (Cañas et al., 1997) and chlorophyll con-tent (Benyas et al., 2013; Chaturvedi et al., 2011). Previously de-scribed significant correlations also include chlorophyll content and soluble proteins (Cañas et al., 1997), leaf size (Ramírez-Valiente et al., 2001) and carotenoids (Benyas et al., 2013). As far as we know, all other correlations are here reported for the first time.

Conclusions

The results presented in this work provide insight into the morphological and biochemical characteristics of five commonly used MAP's. If, by one hand, considerable differences can be observed, both on the leaf characteristics and on their composition between plant species, it is safe to say that all studied plants are rich sources of important bioactive compounds. Indeed, and regarding not only phenolics but also photosynthetic pigments, sugars and starch, the content on the analyzed plants was, in a large extent, higher than previous reports, which may indicate that cultivated plants are better sources of these compounds than wild ones. Furthermore, the obtained data gives a clear characterization of commercially available plants, ready-to-use and actually used by consumers, rather than a description of wild samples, more vulnerable to the influence of abiotic and biotic stresses on their morphological and biochemical characteristics.

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Authors' contributions:

IO redaction of manuscript, data analysis; TP data analysis, experimental design and supervision; MF laboratory work; EB data analysis, experimental design; HF laboratory work; CC data analysis, experimental design; BG field work and data collection, data analysis, experimental design and supervision.

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