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Variation in phenolic, antioxidant and vitamin amounts among some medicinal plants and investigation by PCA analysis: Lamiaceae family

[Variación en las cantidades de fenoles, antioxidantes y vitaminas entre algunas plantas medicinales e investigación por análisis PCA: familia Lamiaceae]

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Abstract: Aromatic and medicinal plants are of great importance to determine the contents of the active compounds of plant origin and to evaluate them depending on variety and climate factors in order to determine the phenolic, antioxidant enzyme activity, vitamin contents in species belonging to the Lamiaceae family. Examination of the characteristics of different species, the highest peroxidase (POD) enzyme activity, ascorbate peroxidase (AxPOD), total antioxidant (TA), malondialdehyte (MDA), caffeic acids (CA), vitamin C contents, and chloric acid (ChA) were obtained in the *M. longifolia* species. The highest vitamin E and catalase (CAT) were determined in the *S. hortensis* species but the highest total phenolic (TP), superoxide dismutase (SOD) enzyme, hydrogen peroxide (H_2O_2) and chlorogenic acid (ChgA) were determined in the *S. spicigera* species. As a result of PCA analysis, it can be said that *Mentha longifolia* (L.) Hudson and *Satureja spicigera* species have significant value in terms of biochemical and phenolic content.

Keywords: Antioxidant; Lamiaceae; Medicinal plants; Phenolic contents; Principal component analysis (PCA)

Resumen: Las plantas aromáticas y medicinales son de gran importancia para determinar el contenido de los compuestos activos de origen vegetal y evaluarlos en función de la variedad y factores climáticos con el fin de determinar la actividad enzimática fenólica, antioxidante, contenido vitamínico en especies pertenecientes a la familia Lamiaceae. El examen de las características de diferentes especies, la mayor actividad enzimática de peroxidasa (POD), ascorbato peroxidasa (AxPOD), antioxidante total (TA), malondialdehído (MDA), ácidos cafeicos (CA), contenido de vitamina C y ácido clorhídrico (ChA) se obtuvieron en la especie *M. longifolia*. La mayor cantidad de vitamina E y catalasa (CAT) se determinó en la especie *S. hortensis*, pero la mayor cantidad total de enzima fenólica (TP), superóxido dismutasa (SOD), peróxido de hidrógeno (H_2O_2) y ácido clorogénico (ChgA) se determinó en la especie *S. spicigera*. Como resultado del análisis de PCA, se puede decir que las especies *Mentha longifolia* (L.) Hudson y *Satureja spicigera* tienen un valor significativo en términos de contenido bioquímico y fenólico.

Palabras clave: Antioxidante; Lamiaceae; Plantas medicinales; Contenido fenólico; Análisis de componentes principales (PCA)

INTRODUCTION

The demand for medicinal and aromatic plants increases almost daily. These aromatic plants play a very important role in living things in ecosystems as a marker of health conditions due to changes in environmental conditions (Firenzuoli & Gori, 2007; Jamshidi-Kia *et al.,* 2018). More than 50.000 species of aromatic plants are used in many fields such as cosmetics and pharmaceuticals. Since these plants are generally collected from the wildlife population, they are not homogeneous in content (Huang, 2011; Rafieian-Kopaei, 2012). Medicinal and aromatic plants have different components with their medicinal content and can be used in different fields with their rich content. Different types of seeds, roots, leaves, fruits, flowers, or even the above-ground biomass of these plants, can be used.

The active compounds found as the main agents in the plant provide important insight into the direct or indirect use of such plants (Rasool-Hassan, 2012). Natural antioxidants such as polyphenols and carotenoids found in medicinal and aromatic plants show a wide variety of biological effects as anti-disease and endurance enhancers. It is important to investigate the potential amounts of antioxidants contained in these plants and to reveal differences in species to support application in different uses (Xu *et al.,* 2017). In biological systems, reactive nitrogen (RNS), reactive oxygen (ROS) and nitric oxide radicals are a types of (RNS), depending on the stress conditions, can damage DNA and cause degradations of proteins (Peng *et al.,* 2014; Li *et al.,* 2015).

Healthy living conditions require not only the consumption of vegetables and fruits but also the consumption of plants with high active ingredients of medicinal and aromatic plants in order to avail of the high antioxidant content (Jastrzebski *et al.,* 2007). In the literature, antioxidant capacity and phenolic contents of medicinal and aromatic plants were found to be significantly positive and it was stated that phenolic compounds contributed to increasing antioxidant activity (Song *et al.,* 2010). Studies have shown that some medicinal and aromatic plants from some regions have stronger antioxidant content and that phenolic compounds in these plants contribute significantly to antioxidant activity (Schinella *et al.,* 2002; Dragland *et al.,* 2003; Cai *et al.,* 2004).

Principal component analyses (PCA) is a statistical method that is about passing data represented by a large number of variables to basic components that can explain the total variance with a mathematical transformation. This analysis was performed to explain and interpret these multivariate and highly correlated data sets better (Guei *et al.,* 2005).

The antioxidant, and phenolic contents of medicinal and aromatic plants can vary widely depending on the species. Therefore, it is very important to determine that the differences between these species and the active substances according to their purpose of usage. For this purpose, in the present study, antioxidant, phenolic and some vitamin contents of some species belonging to the *Lamiaceae* family were investigated.

MATERIAL AND METHODS

Materials

Plants used in this study are from regions in eastern Turkey in 2017 were collected. Above-ground parts and flowers were sampled and analyzed as fresh tissue at -40^{o}C. These collected plants were described in Atatürk University. Plants used in this study include some species of the Lamiaceae family *Lavandula angustifolia* Miller., *Melissa officinalis* L., *Nepeta meyeri* Benth., *Origanum majorana* L., *Origanum onites* L., *Origanum syriacum* L., *Origanum vulgare* L., *Rosmarinus officinalis* L., *Salvia officinalis* L., *Salvia sclarea* L., *Satureja cuneifolia* Ten., *Satureja hortensis* L. and *Satureja spicigera* (C. Koch) Boiss. (flowers and leaves); *Lycopus europaeus* L., *Mentha aquatica* L. and *Mentha longifolia* L. (leaves). The leaves and flowers of the plants were separated from each other for analysis. The samples were then weighed and stored at -20 °C for some physiological analyses.

Determination of antioxidant enzymes

To determine the antioxidant enzymes of the Lamiaceae species, plant samples that flowers and leaves, depending on the variety were homogenized with phosphate buffer and frozen. Then these samples were extracted with phosphate buffer containing EDTA+PMSF (fenilmetil sülfonil florit)+PVP (polyvinylpyrolidone). The POD, SOD, CAT, and AxPOD were measured with a spectrophotometer (Sairam & Srivastava, 2002)

Determination of hydrogen peroxide and lipid peroxidation concentration

The hydrogen peroxide (H_2O_2) was measured according to a study of Loreto and Velikova (2001). So, one gram fresh leaf or flower samples were homogenized in tri-chloroacetic acid (TCA) approximately five minutes. Lipid peroxidation was

measured by the content of total 2-thiobarbituric acid reactive substances and then the taken supernatant was recorded at 532 nm on a spectrophotometer (Cakmak and Horst, 1991; Du *et al.*, 2010).

Determination of total phenolic contents

One gram fresh leaf or flower samples of the plants were taken for analysis. Hexane: dichloromethane (1:2) was added to the samples and shaken for one hour. After shaking, the mixture was centrifuged. Acetone, water, and acetic acid were added to the resulting precipitate. The supernatant obtained after a series of treatments were analyzed (Katsube *et al.*, 2004; Spiridon *et al.*, 2011).

Determination of total antioxidant content and carotenoid contents

According to Prieto *et al.* (1999), the total antioxidant content was analyzed. 0.1 ml of sample solution containing flower or leaves of plants was combined in an Eppendorf tube with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The resulting solution was determined by reading on a spectrophotometer at a wavelength of 695 nm. For total carotenoid content, one gram sample was mixed with 30 ml 30% acetone and homogenized. Then a mixture of samples was measured using the spectrophotometer (Lichtentaler & Wellburn, 1985).

Determination of vitamin E and C contents

For the determination of *vitamin E and C* of plant, vitamin E was measured from leaves using the methods reported by Kumar *et al.* (2013), and vitamin C was measured using the methods reported by AOAC (1990).

Determination of chlorogenic, chiroic acids, caffeic, and proanthocyanidin contents

For the determination of chlorogenic, chiroic acids, caffeic, and proanthocyanidin contents of plants, reverse-phase analytical column and a mobile phase were separated. The HPLC conditions were as following: a C18 (250 × 4.6 mm i.d.; 5 µm particle size; 100 Å pore size) column; the mobile phase of the mixture of acetonitrile and 0.5% aqueous phosphoric acid (11.5:88.5 v/v); the flow rate of 1.0 mL min^{-1} and determination wavelength of 327 nm. At the end of the separation process, the extracts were determined by a reading on HPLC (Mocan *et al.*, 2015) and on a spectrophotometer (Hümmer & Schreier, 2008).

Principal component analysis (PCA)

Principal component analysis (PCA) was performed with the aid of XLSTAT software (Addinsoft (2019). XLSTAT statistical and data analysis solution. Boston, USA. **https://www.xlstat.com**

RESULTS AND DISCUSSION

Evaluation of antioxidant enzymes of Lamiaceae species

In this study, CAT, POD and SOD enzyme activities, and AxPOD, MDA, hydrogen peroxidase, TA of some species of the Lamiaceae family were determined. As a result, significant changes were determined between species (Table No. 1). The highest SOD enzyme and H_2O_2 were measured from *Satureja spicigera* (74.38 EU g $leaf^{-1}$, 9.21 µmol g^{-1} fw, respectively) but the highest CAT enzyme was obtained from *Satureja hortensis* (from *Mentha longifolia* (L.) Hudson (1511.00 EU g $leaf^{-1}$). The highest POD enzyme activity, AxPOD, MDA, and TA were obtained from *Mentha longifolia (L.) Hudson* (357.65 EU g $leaf^{-1}$, 34.57 EU g $leaf^{-1}$ - 102.89 nmol g^{-1}, 1173.67 µmol TE per g^{-1} fw, respectively),

When the lowest antioxidant enzyme activity was examined, the lowest CAT enzyme activity and MDA were determined from *N. meyeri*, the lowest SOD enzyme activity, and H_2O_2 were determined from *M. longifolia*. But the lowest POD and AxPOD were determined from *S. spicigera*.

The presence of sufficient compounds such as vitamin C and vitamin A in the plant cell and antioxidants increase plant resistance under stress conditions, while insufficient levels of antioxidants can cause oxidative stress (Valko *et al.*, 2007). Most medicinal-aromatic plants contain antioxidants. Some non-nutritive and anti-nutritive complexes are included in such plants. Therefore, the nutritional status and nutritional properties of the plants examined should be known (Rehman & Adnan, 2018).

ROS detoxification agents in cells include antioxidant enzymes such as catalase, superoxide dismutase, and ascorbate peroxidase. Enzymatic antioxidants serve as defense agents in plants that are resistant to oxidative damage (Verma and Dubey, 2003; Lee *et al.*, 2007). Superoxide dismutase protects plants from the toxicity of reactive oxygen species produced for energy production. As a result of this effect, more toxic superoxide radical is converted to the less toxic hydrogen peroxide (Vangronsveld & Clijsters, 1994). The CAT enzyme

is an enzyme that catalyzes the decomposition of H_2O_2 (Scandalios, 1987).

Similarly, in the present study, the highest total antioxidant content and the lowest hydrogen peroxide value were obtained in the *M. longifolia* plant, which had the highest POD, AxPOD and MDA values.

Table No. 1
Biochemical contents of some species of the Lamiaceae family

Species	POD	CAT	AxPOD	SOD	MDA	TA	H_2O_2
	EU g leaf^{-1}				nmol g^{-1} fw	µmol TE per g^{-1} fw	µmol g^{-1} fw
Lavandula angustifolia Miller.	238.56	1288.91	24.77	36.27	85.20	890.06	2.90
Lycopus europaeus L.	238.02	1019.57	28.22	37.57	64.07	1023.05	3.13
Melissa officinalis L. *subsp. officinalis*	171.33	1046.11	20.85	41.05	64.42	777.42	3.66
Mentha aquatica L.	117.38	973.33	22.47	59.31	60.08	717.87	3.37
Mentha longifolia (L.) Hudson	357.65	1511.00	34.57	32.68	102.89	1173.67	2.09
Nepeta meyeri	93.40	613.85	19.30	72.41	30.11	796.38	8.86
Origanum majorana	122.77	671.62	19.99	48.12	36.63	780.48	4.99
Origanum onites	112.28	727.79	18.91	**50.39**	**38.68**	730.90	4.72
Origanum syriacum	84.30	789.98	18.91	67.12	45.43	627.01	4.25
Origanum vulgare	146.73	1216.67	28.08	74.14	75.10	897.33	4.21
Rosmarium officinalis L.	156.33	896.71	22.47	44.53	51.16	836.81	3.74
Salvia officinalis L.	163.44	1199.24	26.69	52.41	79.46	821.88	2.67
Salvia sclarea	144.86	745.50	21.79	45.23	42.13	835.11	4.44
Satureja cuneifolia	104.28	764.91	16.10	49.42	42.36	634.60	5.19
Satureja hortensis	204.30	1499.05	33.36	65.51	99.32	1027.35	3.33
Satureja spicigera	79.33	699.12	15.54	74.38	34.82	647.53	9.21

POD: Peroxidase, CAT: catalase, AxPOD: ascorbate peroxidase, SOD: superoksid dismutase, MDA: malondialdehyte, TA: total antioxidant, H2O2: hydrogen peroxide

Determination of phenolic and vitamin contents of the Lamiaceae family

The phenolic content of these plants may vary significantly depending on plant species and climatic factors. In some phytochemical studies, the phenolic contents of medicinal-aromatic plants were obtained. These values were determined to be 0.19 to 101.33 mg of GAE g^{-1} DW (Li *et al.*, 2013), 0.38 to 75.71 mg of GAE g^{-1} DW (Gan *et al.*, 2010a) and 0.12 to 59.43 mg of GAE g^{-1} DW and showed significant differences between species (Gan *et al.*, 2010a).

Phenolic compounds in plants are plant metabolites characterized by several phenol groups. Some of these phenolic compounds exhibit reactive properties in neutralizing and chelating free radicals. Phenolic compounds in plants are important sources of antioxidants for different uses, such as in foods. Naturally grown and used sources contain various phenolic compounds with variable antioxidant activity. Because of these contents, the activity of phenolic compounds may be higher than their individual uses. Song *et al.* (2010), found that the phenolic content of the plants ranged between 0.12-59.43 mg GAE g^{-1}. Duran *et al.* (2015) determined that the total phenolics varied significantly and ranged between 0.411-3.337 mg GAE 100 ml^{-1}. As a result of these differences in phenolic compounds in plants, it is stated that plants contain phenolic with multiple biological properties and these contents may vary depending on their antioxidant activities (Shui and Leong, 2002; Petti and Scully, 2009).

Vitamins are regarded as important nutrients in the foods used and fulfill specific functions necessary for health. Vitamins are also active in carbohydrate, fat and protein metabolism, and the regeneration of new cells. Especially vitamin C tissues play a protective role against oxidative stress (Whitney & Rolfe, 2002). However, although there are few studies on this subject, there may be significant differences in vitamin contents depending on the species in general. Plants with high phenolic content are a source of antioxidants. Considering these results, it is important to quantify the total phenolic and phenolic compounds such as chlorogenic, caffeic and chloric acid in plant extracts in order to reveal the beneficial effects of some

medicinal plants (Gorinstein *et al.,* 2004). In other studies in which the content of phenolic substances was determined, the phenolic contents ranged between 0.42-30.27 µg GAE mg^{-1} (Gulati *et al.,* 2012), 20.90 to 83.25 mg GAE g^{-1} (Zengin *et al.,* 2015).

When these results evaluated, significant differences were observed in the phenolic contents of such as *Hedysarum varium, Onobrychis hypargyrea,* and *Vicia truncatula*. In the present research, the determined total phenolic content ranged between 3.90-18.40 mg GA per g^{-1} FW; total carotenoid ranged between 7.34- 28.24 g vit A eq 100^{-1}.

According to the results of the phenolic analysis of some plant species belonging to the Lamiaceae family, significant differences were found between species (Table No. 2). Although the maximum total phenolic and chlorogenic acid were obtained from the *Satureja spicigera* species, caffeic acids, and chloric acid were obtained from the *Mentha longifolia (L.) Hudson* species. The highest total carotenoid and proanthocyanidin amount was obtained from the *Nepeta meyeri*, and *Satureja hortensis species,* respectively. Vitamin C and vitamin E contents of plants were obtained from the highest, *Mentha longifolia (L.) Hudson* species and *Satureja hortensis* species, respectively (Figure No. 1).

Table No. 2
Chemical contents of some species of the *Lamiaceae* family

Species	Total carotenoid	Total phenolic	Chlorogenic acid	Caffeic acid	Chloric acid	Proantocyanidin
	g vit A eq 100^{-1}	mg GA per g^{-1}FW	mg g^{-1} fw			%
Lavandula angustifolia Miller.	9.57	6.34	5.91	1.03	91.73	47.76
Lycopus europaeus L.	11.68	3.92	5.06	1.03	70.81	52.40
Melissa officinalis L. *subsp. officinalis*	11.55	7.82	7.29	0.73	73.13	37.41
Mentha aquatica L.	11.59	5.33	4.93	0.58	40.38	44.47
Mentha longifolia (L.) Hudson	7.34	3.90	5.19	1.92	126.06	50.40
Nepeta meyeri	28.24	10.26	13.23	0.38	40.04	27.84
Origanum majorana	17.03	5.98	7.71	0.51	45.00	32.14
Origanum onites	14.20	8.14	10.85	0.57	57.02	21.42
Origanum syriacum	14.00	6.59	6.08	0.41	32.19	34.83
Origanum vulgare	14.49	6.67	6.16	0.72	50.48	55.59
Rosmarium officinalis L.	11.76	6.60	8.79	0.81	71.53	27.36
Salvia officinalis L.	9.60	4.32	3.99	0.82	50.66	56.78
Salvia sclarea	15.49	5.38	6.94	0.61	50.40	36.31
Satureja cuneifolia	15.33	10.73	10.00	0.43	52.05	25.92
Satureja hortensis	12.00	5.40	4.99	1.02	63.32	70.98
Satureja spicigera	25.43	18.40	17.15	0.32	46.31	22.46

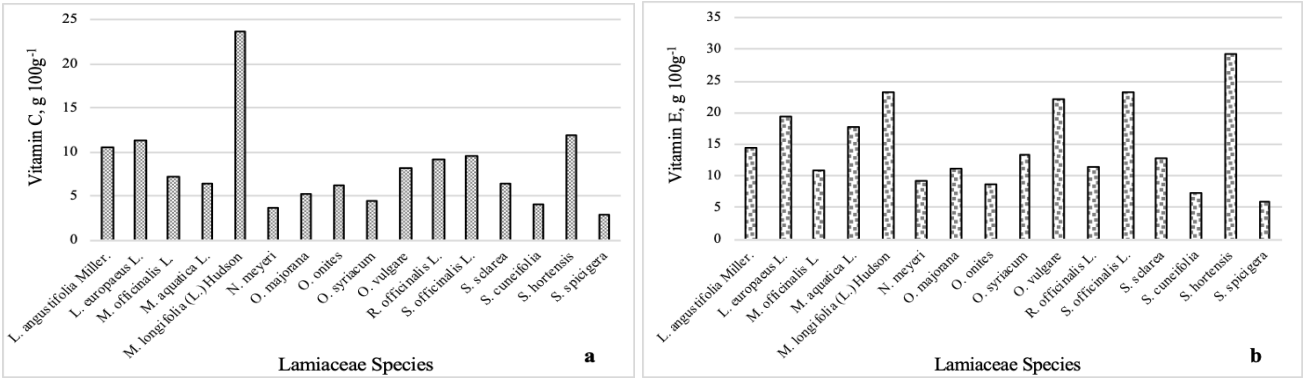

Figure No. 1
Vitamin C (a) and vitamin E (b) contents of *Lamiaceae* species

PCA analysis

As a result of the PCA analysis, PC1 and PC2 explained 89.68% of the total variance for the biochemical content graph and 87.29% for the phenolic contents graph. PC1 explained 74.87% and 69.52% of the total variance alone in biochemical and phenolic contents, respectively (Figure No. 2). PC1 was found to be related to POD, CAT, AxPOD, MDA, TA and H_2O_2 in biochemical contents and was found to be related to total carotenoid, total phenolic, chlorogenic acid, caffeic acid, proantocyanidin, vitamin C and vitamin E in phenolic contents. PC2 was found to be related to SOD in biochemical contents and was found to be related to chloric acid in phenolic contents (Table No. 3).

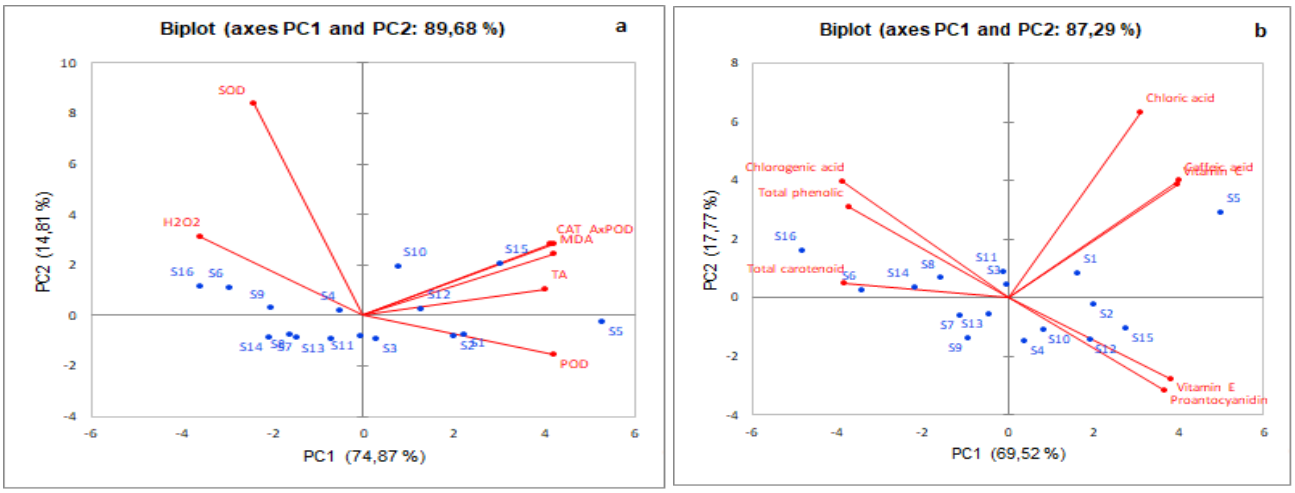

Figure N° 2

PCA graphs of the some Lamiaceae species for biochemical contents (a) and phenolic contents (b)
S1: *Lavandula angustifolia* Miller., S2: *Lycopus europaeus* L., S3: *Melissa officinalis* L. subsp. Officinalis, S4: *Mentha aquatica* L., S5: *Mentha longifolia* (L.) Hudson, S6: *Nepeta meyeri*, S7: *Origanum majorana*, S8: *Origanum onites*, S9: *Origanum syriacum*, S10: *Origanum vulgare*, S11: *Rosmarium officinalis* L., S12: *Salvia officinalis* L., S13: *Salvia sclarea*, S14: *Satureja cuneifolia*, S15: *Satureja hortensis*, S16: *Satureja spicigera*

When PCA graph of the species was examined (Figure No. 2a), Satureja spicigera (S16 in graph) was found to be prominent in terms of H2O2 and SOD, and Mentha longifolia (L.) Hudson (S5 in graph) was found to be prominent in terms of POD. When PCA graph of the species was examined (Figure No. 2b), Satureja spicigera (S16 in graph) was found to be prominent in terms of chlorogenic acid and total phenolic, Mentha longifolia (L.) Hudson (S5 in graph) was found to be prominent in terms of caffeic acid, chloric acid and vitamin C. Therefore, it can be said that these two species (S16 and S5) have significant value in terms of biochemical and phenolic content.

According to the results of antioxidant, phenolic and vitamin analysis of some plant species belonging to the Lamiaceae family, significant content differences were determined depending on the species. In the results of the present study, M. longifolia, S. hortensis and S. spicigera plants were found to be rich in antioxidant enzyme activity, total phenolic content, and vitamins. However, it was determined that the biochemical contents of these plants may vary according to climatic soil and growing conditions and that their contents should be determined before use. It has been attempted to explain the information in a multivariate numerical data set with fewer variables with minimum information loss. PCA analysis was performed to explain and interpret these multivariate and highly correlated data sets better (Guei et al., 2005). As a result of PCA analysis, it can be said that Mentha longifolia (L.) Hudson and Satureja spicigera species have significant value in terms of biochemical and phenolic content.

Table No. 3
Factor loadings of PCA analysis

	PC1	PC2
Biochemical contents		
POD	**0.942**	-0.154
CAT	**0.926**	0.280
AxPOD	**0.937**	0.282
SOD	-0.541	**0.830**
MDA	**0.938**	0.242
TA	**0.896**	0.104
H_2O_2	**-0.802**	0.310
Phenolic contents		
Total carotenoid	**-0.850**	0.056
Total phenolic	**-0.822**	0.346
Chlorogenic acid	**-0.859**	0.441
Caffeic acid	**0.888**	0.445
Chloric acid	0.686	**0.708**
Proantocyanidin	**0.813**	-0.353
Vitamin C	**0.884**	0.429
Vitamin E	**0.851**	-0.309

POD: Peroxidase, CAT: catalase, AxPOD: ascorbate peroxidase, SOD: superoksid dismutase, MDA: malondialdehyte, TA: total antioxidant, H_2O_2: hydrogen peroxide

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