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Numerical Comparison of Anatomical Features of Lamiaceae Taxa Named After Spil Mountain

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Abstract: In this study, the anatomical features of some taxa (*Origanum sipyleum*, *Satureja parnassica* subsp. *sipylea*, *Sideritis sipylea*, *Thymus sipyleus* subsp. *sipyleus* var. *sipyleus*) belonging to the Lamiaceae distributed on the Spil Mountain of Turkey were compared statistically. Lamiaceae family to which the investigated taxa belong are of high importance for their antioxidant potential, multiple pharmaceuticaluses in folk medicine. The some anatomical features of the stem were selected and measurements were taken. The data obtained from the anatomical features were evaluated statistically. By this study it has been determined that cortex length and diameter of pith are the best character pairs which represent the variations in them. Thus, the cortex length and diameter of pith can be viewed as a taxonomic character for classification of species. The results showed that investigated taxa also could be distinguished from each other not only by their morphological features but numerical anatomical characters as well.

Keywords: Anatomy, Lamiaceae, Numerical analysis, Statistical analysis

Adını Spil Dağı'ndan Alan Lamiaceae Taksonlarının Anatomik Özelliklerinin Sayısal Olarak Karşılaştırılması

Öz: Bu çalışmada, Türkiye'nin Spil Dağı'nda yayılış gösteren Lamiaceae'ye ait bazı taksonların (*Origanum sipyleum*, *Satureja parnassica* subsp. *sipylea*, *Sideritis sipylea*, *Thymus sipyleus* subsp. *sipyleus* var. *sipyleus*) anatomik özellikleri istatistiksel olarak karşılaştırıldı. Çalışmada incelenen taksonların da ait olduğu Lamiaceae familyası, antioksidan potansiyelleri ve halk hekimliğinde çoklu farmasötik kullanımları nedeniyle büyük önem taşımaktadır. İncelenen taksonların bazı anatomik gövde özellikleri seçilerek bunlardan anatomik ölçümler alındı. Anatomik özelliklerden elde edilen veriler istatistiksel olarak değerlendirildi. Bu çalışma ile korteks uzunluğu ve öz çapının, varyasyonları temsil eden en iyi karakter çiftleri olduğu belirlenmiştir. Böylece, korteks uzunluğu ve öz çapı, türlerin sınıflandırılması için taksonomik karakterler olarak görülebilir. Elde edilen sonuçlar, incelenen taksonların sadece morfolojik özellikleriyle değil, sayısal anatomik karakterleri ile de birbirlerinden ayırt edilebildiğini göstermiştir.

Anahtar Kelimeler: Anatomi, Lamiaceae, Sayısal analiz, İstatiksel analiz

1. Introduction

Spil Mountain which is the natural distribution area of the studied taxa, is located west of Turkey starts from the 60 m level of the Gediz River plain and reaches a height of 1517 meters. The investigated taxa take their names from the mountain. Lamiaceae family to which the taxa belong are of high importance for their antioxidant potential, multiple pharmaceutical uses in folk medicine, and commercial essential oils as well as their wide cosmetic and culinary applications. More specifically, the genera *Origanum* and *Satureja* have found a wide

variety of pharmaceutical and food applications (Formisano et al., 2014). The genus *Origanum*, as an important ethnomedicinal plant, is found in the Mediterranean region, North Africa, and Eurasia (Loizzo et al., 2009). The genus *Satureja* including about 200 species mainly grows in West Asia, Middle East, Europe, North Africa, Mediterranean region, and South America. *Sideritis* L. comprises more than 150 annual and perennial species and subspecies worldwide (Gonzalez et al., 2011) and most of them are to be found as wild-growing plants in countries around the Mediterranean Basin and the Caspian Sea (Barber et al., 2002); many among them are confined to small geographical areas being exclusive endemics either to single islands (e.g. Crete, Greece; Canary islands, Spain), single countries (e.g. Spain, Morocco, Italy, Greece, Turkey etc.) or a couple of adjacent countries e.g. Balkans or Greece, and Turkey (Charalampia-Kloukina et al., 2020). The genus *Thymus* L. consists of about 215 species of herbaceous perennials and subshrubs. The Mediterranean region can be described as the center of the genus (Vouillamoz and Christ, 2020).

Most previous studies have revealed the chemical composition and antioxidant capacity of some of the species in the Lamiaceae family including *Origanum* (Loizzo et al., 2009), *Satureja* (Kemertelidze et al., 2012; Ghasemi et al., 2014; Moradi and Sadeghi, 2017). *Thymus* (Tohidi et al., 2017; Bahman et al., 2005). Anatomical studies of Lamiaceae have been carried out by many authors (Atasagun et al., 2015; Çelep et al., 2011); however, anatomical studies carried out on the investigated taxa were limited with several authors (Ozer et al., 2017). We have not found any studies on statistical comparison of numerical anatomical features, such as this one we have just done.

In this study, the stem anatomical properties of *Origanum sipyleum* L. *Satureja parnassica* subsp. *sipylea*, *Sideritis sipylea*, *Thymus sipyleus* subsp. *sipyleus* var. *sipyleus* species belonging to Lamiaceae, which are localized Spil Mountain in Turkey, were investigated. The anatomical variations in the taxa have been investigated by means of numerical methods. Results were supported by tables and graphs. The purpose was to determine statistically the clonseness of the taxa with the help of numerical anatomic characters.

2. Material and Method

The plant samples were collected from Spil Mountain in the western Anatolian region of Turkey where they grow naturally (Figure 1A-D). They were collected in 2019 during their flowering period, from March to August. The research was primarily completed in three stages: field study, where plants were taken, laboratory studies where

anatomical studies were performed, and statistical studies in which the obtained results were evaluated mathematically. The plant samples were fixed in 70% ethyl alcohol (C_2H_5OH). For microscopic observations, sections were taken from the stem parts of the plant and were stained using safranin and fastgreen dyes (Bozdağ et al., 2016). Preparations prepared from these sections were examined using Leica DM3000 motorized microscope lenses. Some stem anatomical characters (epidermis, cortex diameter, tracheal diameter, pith diameter) were selected for the numerical analysis. Measurements were made in these structures. Minimum, maximum, average and standart error values were determined and these values were turned into tables. Characters were coded as 1- 6 and the taxa were coded as A-D (Table 1). Significance of the differences between the taxa and characters were evaluated by Analysis of variance and Pearson's correlation. Statistical analysis were performed using the MINITAB software package.

Figure 1. Natural photos *Origanum sipyleum* (A), *Satureja parnassica* subsp. *sipylea* (B), *Sideritis sipylea* (C), *Thymus sipyleus* subsp. *sipyleus* var. *sipyleus* (D)

Table 1. Codes of the examined taxa and anatomical features.

The examined taxa	Code	Anatomical features	Code
Origanum sipyleum	**A**	Epidermis length	**1**
Satureja parnassica subsp. *sipylea*	**B**	Epidermis width	**2**
Sideritis sipylea	**C**	Cortex length (µm)	**3**
Thymus sipyleus subsp. *sipyleus.* var. *sipyleus*	**D**	Cortex width (µm)	**4**
		Diameter of tracheal elements (µm)	**5**
		Diameter of pith	**6**

3. Results and Discussion

3.1. Anatomical findings:

Origanum sipyleum: The stem cross-section is in the form of a protruding circle or it has squared similar to round shape. Epidermis cells are fragmented, oval and rectangular in shape. Below the epidermis are large-diameter parenchyma cells with thickened walls as collenchyma tissue. The phloem region is prominent and located just above the xylem. Xylem elements take up a very large area. There is a large pith cavity in the center of the stem and smooth circular pithy parenchyma cells cover this cavity (Figure 2A-B).

Satureja parnassica subsp. *sipylea*: Cross-section of the stem is 4-cornered. Epidermis cells of stem are oval, rectangular or square shaped. It is composed of a single cells, and covered with a thick and undulated cuticle. There are 5-8 rows of cortex parenchyma. Between the cortex layer, there is a cell layer with thickened walls consisting of a single row of rings. The collenchyma tissue, located on the corner stem. The cambium is not distinguishable. The region of pith occupies a large area in the cross-section. (Figure 2C-D).

Sideritis sipylea: Cross-section of the stem is 4-cornered. Collenchyma is seen in corners as wide and shallow. Epidermis cells are oval, rectangular or nearly square in shape. There are many non-glandular and glandular hairs on the epidermis. At the corners of the stem, there are cells collenchyma and under the epidermis, there are cells with chlorophyll consisting of 1-8 cell lines. Below these cells, there are the cortex parenchyma of 1-3 rows rectangular and oval in shape. The phloem area is clearly and there are clusters of scleranchyma cell on it. 2-3 rows of cambium are found at the stem. The region of pith is wide in the center and consists of circular parenchyma cells (Figure 2E-F).

Thymus sipyleus subsp. *sipyleus* var. *sipyleus*: Cross-section of the stem has squared shape. Epidermis cells are oval, rectangular or nearly square in shape. There is a thin cuticle layer on the epidermis. There are many non-glandular and glandular hairs on the epidermis. In the corners of the stem, there is a cluster of collenchyma cells.

Under these cells, there are cells of cortex parenchyma of 1-5 rows are rectangular and oval in shape. The phloem region is not evident. There is a scleranchyma ring on elements of vascular. The xylem elements are very distinct and the trachea are sometimes irregularly arranged. The pith region in the center of the stem is wide and consists of circular parenchyma cells (Figure 2G-H).

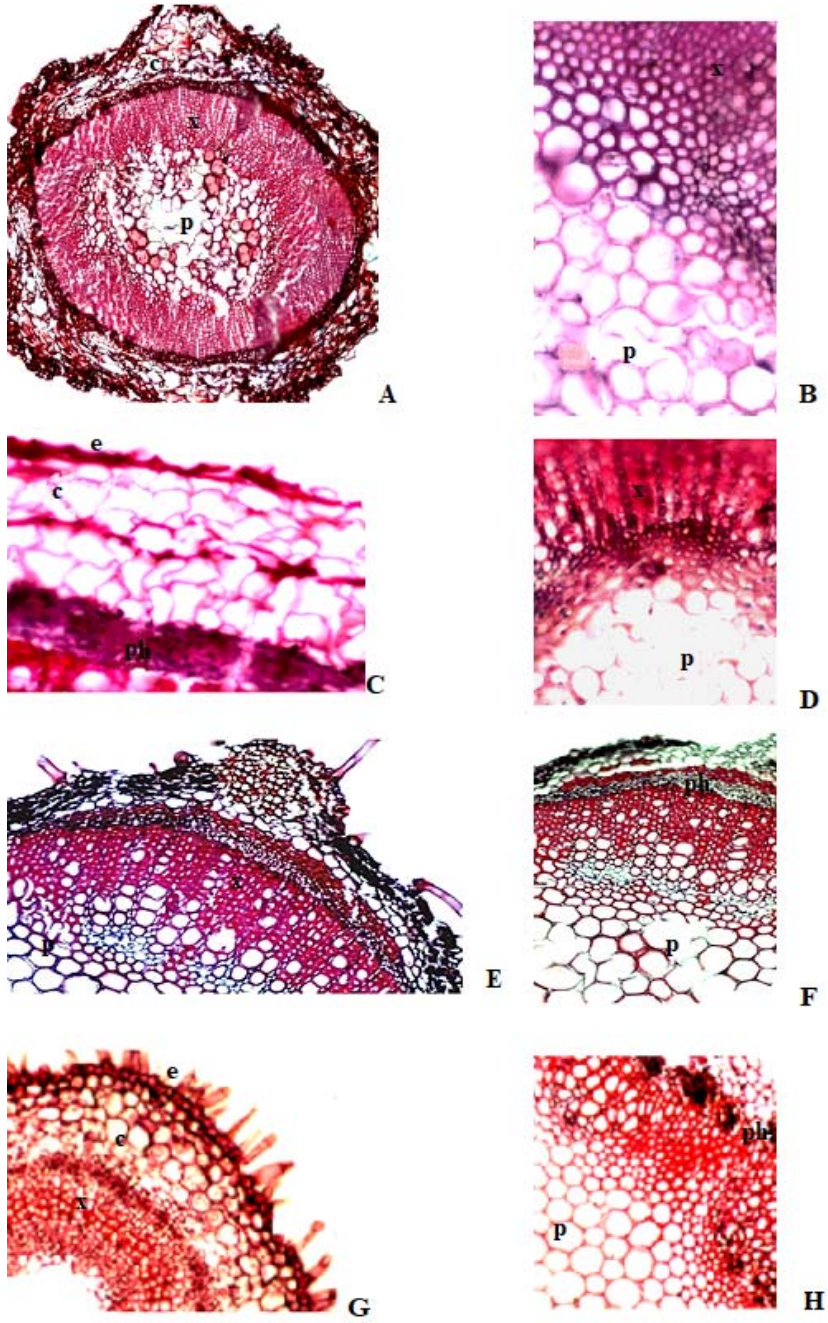

Figure 2. Stem cross-section of taxa

A-B: *Origanum sipyle* C-D: *Satureja parnassica* subsp. *sipylea* E-F: *Sideritis sipylea* G-H: *Thymus sipyleus* subsp. *sipyleus* var. *sipyleus* (Microscope objective numbers: A:x4; B, D, G: x10; C, E, F, H:x20) e: epidermis cells, x:xsilem, c:cortex layer; p:pith, ph:phloem

3.2. Results of statistical analysis: The anatomical measuments of the investigated taxa were shown in Table 2. Significance of the differences between the investigated taxa and anatomical characters was evaluated by analysis of variance and pearson correlation. While the results between the taxa examined according to the pearson correlation method are given in Table 3, the results obtained according to the variance analysis method are given in Table 4. According to both methods of statistical analysis, there are the statistical differences among (*O. sipyleum-S. parnassica* subsp. *sipylea*) and (*O. sipyleum-S. sipylea*) in the values 0.005; 0.005; 0.001 and 0.002 respectively at level of 0,01** and 0.05* (Table 3-4). According to both statistical methods, it was determined that the taxon encoded with D *Thymus sipyleus* subsp. *sipyleus.* var. *sipyleus* was not statistically significant with the other taxa studied. The fact that the taxon is not close to the other taxa systematically examined shows that it confirms the statistical results. As observed in laboratory studies also, *Thymus sipyleus* subsp. *sipyleus* var. *sipyleus* that encoded with D differs from other investigated taxa due to the different anatomical features such as the unclear phloem region.Thus, this result supports the statistical results. When the anatomical features were evaluated with both statistical analysis methods, there were differences between the characters (cortex length-cortex width), (cortex length-diameter of pith) and (cortex width-diameter of pith) in the values 0.001; 0.018; and 0.017 respectively at level of 0,01** and 0.05* (Table 4-5). On the other hand, according to method of pearson correlation, there are also the differences between epidermis length and diameter of tracheal in the value 0.029 at level of 0.05* (Table 5). According to result of variance analysis, significant differences also were found between (cortex length-diameter of tracheal) and (cortex width-diameter of tracheal) in the values 0.047 and 0.050 respectively at level of 0.05* (Table 6).

The examined taxa carry the characteristics of the family they belong to (Lamiaceae). The researchers emphasized that the typical feature of the family is a four-cornered body and a well-developed collenchyma tissue at the corners of the body as a support tissue (Baran and Özdemir, 2009). Similiar features were also seen in the anatomical structures of the studied taxa. While a collenchyma layer is observed at the corners all of the investigated taxa stem, while this layer shows a different distribution on the body in taxa.

However, any extraxilar sclerenchymatic tissue is not seen in stem the studied taxa except to *Sideritis sipylea*, as not seen in *L. lycium* and in *L. moschatum* var. *rhodium*, either (Baran and Özdemir,

2009; 2011). Many genera of Lamiaceae, even annual taxa, usually bear extraxilar sclerenchymatic tissues in their stems (Dinç et al., 2008).

By the analysis of the investigated taxa from 6 stem anatomy related characters, it has been determined that cortex length and diameter of pith are the best character pairs which represent the variations in them. Thus, the cortex length and diameter of pith can be viewed as a taxonomic character for classification of species.

In this study, four Lamiaceae taxa that spread in Spil Mountain compared statistically regarding anatomical characters. The anatomical variations in the taxa have been investigated by means of numerical methods. Similar statistical study has been carried out on some *Salvia* L. belong to family Lamiaceae by some researchers (Özdemir et al., 2016). On the other hand, similar to the results of our study, statistically significant results were obtained in studies on numerical anatomical features of plants belonging to different families (Özdemir, 2018). We aimed to find statistical and anatomical differences in addition to morphological characters to distinguish these taxa. The results showed that investigated taxa also could be distinguished from each other not only by their morphological features but numerical anatomical characters as well. By the analysis of the investigated taxa from some anatomy related characters, it has been also found that the results from numerical analysis of anatomy characters can provide additional evidences, which correspond to the anatomy for the recognition of the taxa.

Table 2. Measurement results of some stem anatomical structures of examined the taxa.

	Origanum sipyleum		***Satureja parnassica* subsp. *sipylea***	
	Min-Max	**Mean±SD**	**Min-Max**	**Mean±SD**
Epidermis width (µm)	05.29-10.58	07.13±02.41	10.70-14.00	12.35±06.20
Epidermis length (µm)	10.25-26.45	18.50±07.90	15.24-25.45	15.25±08.20
Cortexwidth (µm)	06.25-12.55	09.12±05.06	10.24-15.20	12.56±07.88
Cortex length (µm)	10.10-15.87	12.56±07.88	03.54-35.00	19.50±09.60
Diameter of tracheal elements (µm)	11.14-37.00	24.12±09.82	11.40- 32.25	21.35±09.45
Diameter of pith (µm)	26.45-84.64	55.56±17.80	10.50-52.90	31.5±12.80
	Sideritis sipylea		***Thymus sipyleus* subsp. *sipyleus.* var. *sipyleus***	
	Min-Max	**Mean±SD**	**Min-Max**	**Mean±SD**
Epidermis width (µm)	12.70-13.70	12.13±06.21	10.59-10.70	10.03±08.40
Epidermis length (µm)	16.70-18.70	17.50±07.80	15.37-37.80	27.10±11.40
Cortex width (µm)	10.24-15.29	12.56±07.48	11.53-26.47	17.05±08.06
Cortex length (µm)	15.70-23.80	19.50±08.18	15.60-43.70	28.05±10.40
Diameter of tracheal elements (µm)	10.40- 42.35	26.38±11.45	05.21-21.16	12.10-04.82
Diameter of pith (µm)	15.80-63.48	39.5±12.88	10.50-15.87	12.50±08.60

Min: Minimum, Max: Maksimum, SD: Standard Deviation

Table 3. Pearson correlation based on the investigated taxa.

	A	B	C
B	0.940		
	0.005*		
C	0.986	0.972	
	0.001**	0.001**	
D	0.142	0.235	0.200
	0.789	0.654	0.704

Significant at the level of P< 0.05. ** Significant at the level of 0.01. Probability: P value; Abbreviations: A-D: Codes of investigated taxa, A: *Origanum sipyleum*; B: *Satureja parnassica* subsp. *sipylea* C: *Sideritis sipylea*; D: *Thymus sipyleus* subsp. *sipyleus.* var. *sipyleus*

Table 4. Correlation between taxon (Analysis of Variance).

Source	MS	F-value	Probability	Significance
A-B	242.9	30.36	0.005	**
A-C	135.9	81.77	0.018	**
A-D	5.520	0.080	0.761	NS
B-C	357.5	27.00	0.002	**
B-D	90.03	43.40	05.43	NS
C-D	21.40	17.30	0.704	NS

MS: Mean Square; *P<.05; **P<.01; A-D: Codes of taxon; NS: Not Significant; Probability: P value A: *Origanum sipyleum*; B: *Satureja parnassica* subsp. *sipylea* C: *Sideritis sipylea*; D: *Thymus sipyleus* subsp. *sipyleus.* var. *sipyleus*

Table 5. Pearson correlation based on anatomical characters.

	1	2	3	4	5
2	0.296				
	0.704				
3	0.518	0.637			
	0.482	0.363			
4	0.477	0.671	0.999		
	0.523	0.329	0.001**		
5	0.029*	0.802	0.792	0.815	
	0.971	0.198	0.208	0.185	
6	0.468	0.618	0.982	0.983	0.863
	0.532	0.382	0.018*	0.017*	0.137

Significant at the level of P< 0.05. ** Significant at the level of 0.01; Probability: P value Abbreviations: A-D: Codes of natomical features

1: Epidermis length
2: Epidermis width
3: Cortex length (µm)
4: Cortex width (µm)
5: Diameter of tracheal (µm)
6: Diameter of pith

Table 6. Correlation between anatomical features (Analysis of Variance)

Source	MS	F-value	Probability	Significance
1-2	1.48	0.19	0.704	NS
1-3	4.51	0.71	0.485	NS
1-4	3.81	0.59	0.525	NS
1-5	0.12	0.01	0.910	NS
1-6	3.60	0.52	0.561	NS
2-3	31.1	1.37	0.368	NS
2-4	35.6	1.62	0.321	NS
2-5	50.0	3.64	0.198	NS
2-6	30.0	1.16	0.382	NS
3-4	31.5	90.0	0.001	**
3-5	37.8	28.1	0.047	*
3-6	30.6	53.1	0.018	*
4-5	74.5	74.3	0.050	*
4-6	90.2	57.0	0.017	*
5-6	83.13	5.24	0.137	NS

MS: Mean Square; *P<.05; **P<.01;

1-6: Codes of anatomical features; NS: Not Significant.

1: Epidermis length
2: Epidermis width
3: Cortex length (µm)
4: Cortex width (µm)
5: Diameter of tracheal (µm)
6: Diameter of pith

As a result, we believe that the study provides a new comparing opportunity for future researchers on the related subjects.

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