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Mathematically Comparison of Anatomical Features of Endemic Phlomis Russeliana (Sims.) Bentham and Phlomis Armeniaca Willd. (Labiatae) from Turkey

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Abstract

Lamiaceae families to which the investigated 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. In this study, the anatomical features of endemic Phlomis russeliana (Sims.) Bentham and endemic Phlomis armeniaca Willd. Belonging to the Lamiaceae were compared mathematically. The numerical data obtained from the laboratory studies were tried to be tested by comparing the features of the species. These comparisons are supported numerically by statistical analysis. The results showed that investigated species also could be distinguished from each other not only by their morphological features but numerical anatomical characters as well.

Keywords: Mathematically comparison; Anatomy; Phlomis; Statistical

Abbreviations: MS: Mean Square; NS: Not Significant; CU: Cuticle; PP: Palisade; SP: Sponge; AB: Abaxial epidermis.

Introduction

Lamiaceae families 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 [1]. Phlomis, large genus of Old World has aromatic herbs, subshrubs and shrubs which often bear woolly leaves. This plant forms an excellent weed-smothering ground cover. The flowered stems dried make pretty winter decorations. Aromatic herbs and shrubs which have flowers resembling the lips of a mouth and four-lobed ovary, of which each lobe yields a seed are grown primarily for their dense whorls of lipped flowers and attractive foliage. The leaves often wooly are highly variable in size and shape, though they are neatly arranged in opposing pairs. The flowered stems borne at shoot tips mostly in spring and in summer can be quite tall. The spaces among the usually ball-like clusters of flower buds that open into large, colorful, highly irregular flowers in lengthy succession are tight. Each flower has a hooded upper lip and a more open, spreading lower lip, much like some Salvia species. These are rugged and attractive plants for sunny sites [2-6].

Anatomical studies of Lamiaceae have been carried out by many authors; however, anatomical studies carried out on the investigated taxa were limited with several

authors [7-9]. 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 anatomical features of endemic Phlomis russeliana (Sims.) Bentham and Phlomis armeniaca Willd. Species belonging to Lamiaceae, which are localized the northern and western Anatolia 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 is to determine statistically the clonseness of the taxa with the help of numerical anatomic characters.

Material and Methods

The research was primarily completed in three stages: field study, where plant were taken, laboratory studies where anatomical studies were performed, and statistical studies in which the obtained results were evaluated mathematically. The plant samples were collected from the northern and western Anatolian part of Turkey where they naturally grow. For anatomical investigations, the parts of the species were fixed in 70% ethyl alcohol. For microscopic observations, sections were taken from the parts of the plant and were stained using saffron and fast green dyes. Preparations prepared from these sections were examined using Leica DM3000 motorized microscope. The anatomical features were selected and measurements were made in these structures. The minimum, maximum, average and standard error values of the size of the cells belonging to the parts of each taxon were determined. The numerical data's of the anatomical the taxa examined were evaluated statistically by Pearson correlation analysis, One-way ANOVA and Variance analysis. The anatomical properties were coded as 1-12 (Table 1).

Code		*Phlomis russeliana*		*Phlomis armeniaca*	
		Min-Max	**Mean±SD**	**Min-Max**	**Mean±SD**
Root					
Epidermis width (µm)	1	06,67-23,33	15,57 ± 05,10	10,17-36,67	23,67 ± 07,82
Cortex parenchyma (µm)	2	16,67-33,33	25,50 ± 05,20	23,37-50,00	37,10 ± 07,62
Endodermis width (µm)	3	08,33-10,33	09,16 ± 00,68	13,33-23,43	18,46 ± 03,58
Diameter of tracheal elements (µm)	4	05,10-10,33	06,86 ± 01,58	11,67-23,33	17,40 ± 03,61
Stem					
Epidermis width (µm)	5	08,67-23,33	16,51 ±04,10	13,33-21,30	18,33 ±03,46
Cortex parenchyma (µm)	6	06,67-33,33	18,10 ± 07,32	30,27-66,67	48,10 ± 10,13
Diameter of tracheal elements (µm)	7	03,33-08,33	05,45 ± 01,68	05,33-08,20	06,92 ± 00,83
Diameter pith (µm)	8	13,33-60,33	37,06 ± 16,38	30,33-126,70	89,10 ± 26,55
Leaf					
Epidermis width (µm)	9	13,33-23,33	23,11± 07,40	16,30-36,13	26,10± 06,60
Palisade parenchyma (µm)	10	16,67-23,30	16,90 ± 01,99	14,10-27,30	21,70 ± 03,55
Spongy parenchyma (µm)	11	16,63-30,23	23,75 ± 07,48	20,53-41,23	30,75 ± 06,77
Diameter of tracheal elements (µm)	12	04,67-09,33	06,96 ± 01,48	05,17-09,78	07,16 ± 01,12

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

Table 1: Measurement results of some anatomical structures of examined the species.

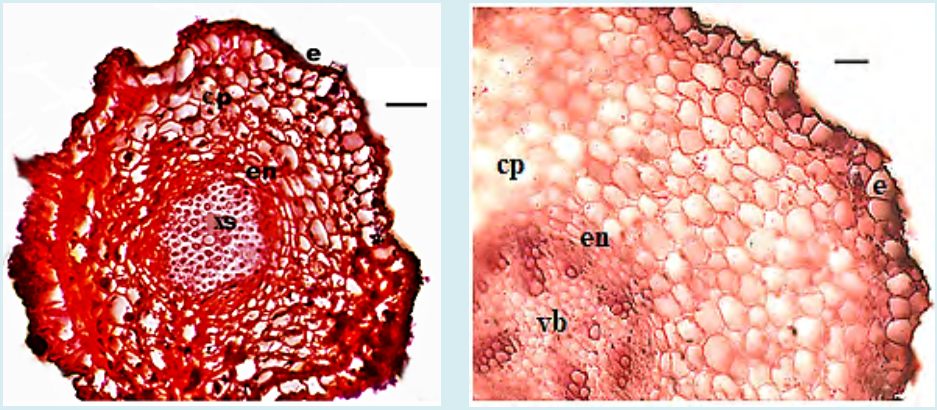
Results and Discussion

Anatomical Results

The root sections of the investigated taxa have a round shape. There is a epidermis protective tissue in outermost on the part of the sections. There are cortex layer under the epidermis layer Cortex layer covers a larger area in P. armeniaca. Its cell row is between 5 and 9. P. russeliana has the cortex layer with 4-7 cell row. Radial vascular bundles are clearly observed in root cross- sections of both species. Vascular bundles are located under the cortex layer. In P. russeliana, this layer with cells of xylem continues to the center of cross section region. Also, parenchymatic cells

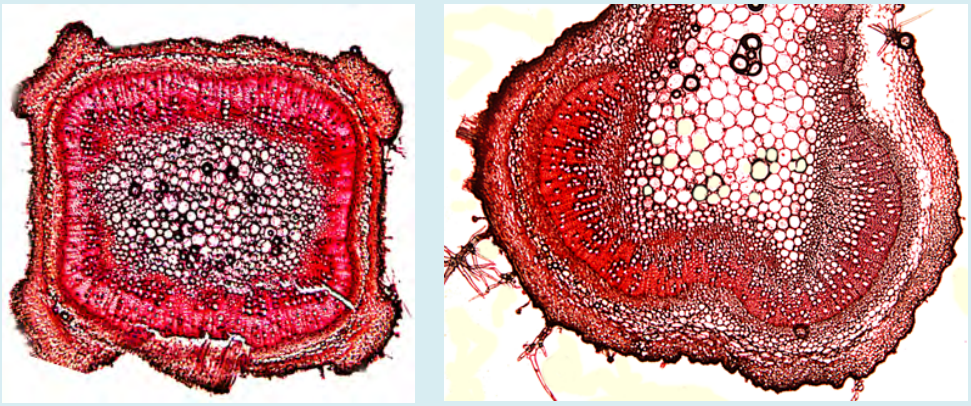
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are not observed in the center region. In the other species, parenchymatic cells are located between the bundles and in the center. Also these cells can extend to the center of the section. They show a radial arrangement from the center region towards the outside of the section. The cambium cells cannot be seen in root cross sections of both species (Figure 1).

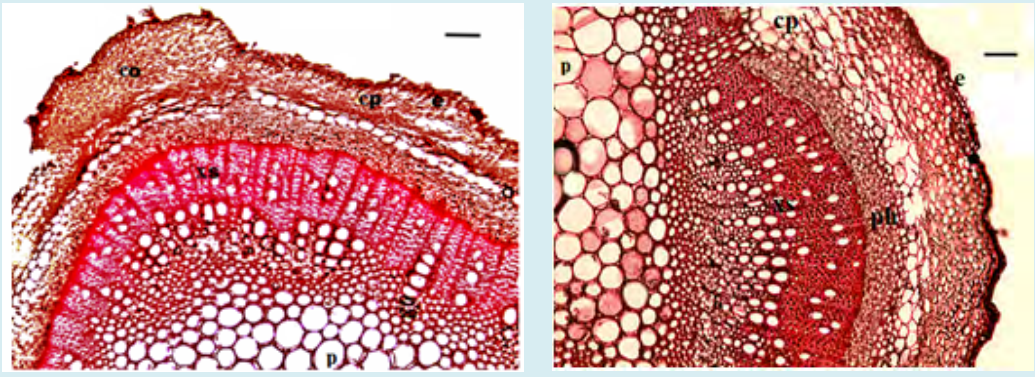


A B

Figure 1: Root cross sections Phlomis russeliana (A), Phlomis armeniaca (B)(e- Epidermis; cp- Cortex; en- Endodermis; xs- Xylem; v- Vascular bundle) (Scale bar: 50µm).



A C



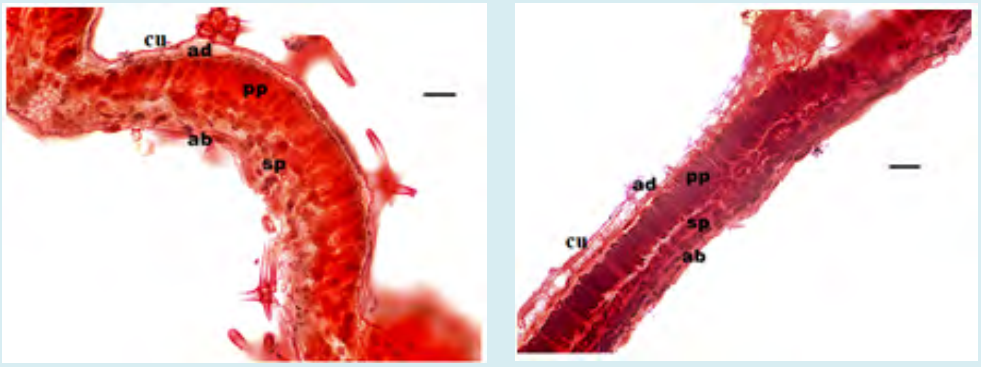
B D

Figure 2: Stem cross sections- Phlomis russeliana (A,B) Phlomis armeniaca (C,D) (e- Epidermis; c- Collenchyma; cp-Cortex; xs- Xylem; ph- Phloem; pi- Pith) (Scale bar: 100µm).

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The stem cross-section of P. russeliana is 4-cornered. The stem cross- section of the P. armeniaca, unlike the P. russeliana, is collapsed inwards from the edges. This species differs from other species in this aspect. There are a layer of cuticle on epidermis cells of both species. Epidermis cells are oval, rectangular or nearly square in shape. There are many aglandular and glandular hairs on the epidermis of both species. At the corners of the stems, there are cells collenchyma and under the epidermis, there are cells with chlorophyll consisting. Below these cells, there are the cortex parenchyma. The cortex parenchyma cells have 5-9 rows at P. russeliana, while P. armeniaca is in 7-10 rows. 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 of both species and consists of circular parenchyma cells (Figure 2).

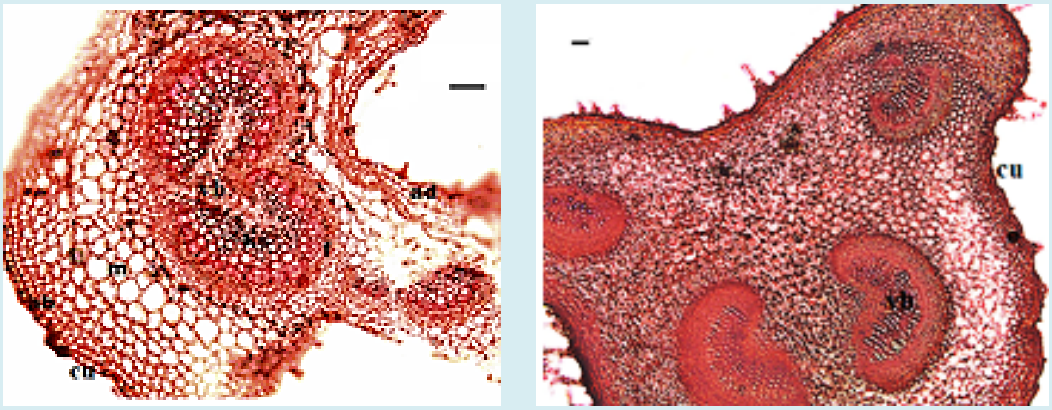
Outside the adaxial and abaxial sides of the cross sections taken from the leaves of both species, there is the cuticle layer, beneath it, the single row epidermis cells, and the stoma cells. There are vascular bundles lined up at certain intervals in the mesophile layer. Mesophyll layer of the leaf are separated as palisade and spongy parenchyma (Figure 3).



A B

Figure 3: leaf cross sections- Phlomis russeliana (A), Phlomis armeniaca (B) (cu- Cuticle; ad- Adaxial epidermis; pp- Palisade; sp-Sponge; ab- Abaxial epidermis, (Scale bar: 50µm).

When the cross-sections taken from the petiole of the species are examined; both species have a layer of cuticle on the adaxial and abaxial faces. The epidermis layers of both species are in a single row. Parenchymatic cells following the epidermis layer in P. armenica have 4-5 rows of corner collenchyma thickening. The latter cells are typical thin-walled, round cells. Parenchymatic cells on the abaxial face are more in P. russelina than in the adaxial face (Figure 4).



A B

Figure 4: Petiol cross sections- Phlomis russeliana (A), Phlomis armeniaca (B)(cu- Cuticle; ad- Adaxial epidermis; ab- Abaxial epidermis; VB- Vascular bundle, (Scale bar: 100µm).

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The examined species 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. Similar features were also seen in the anatomical structures of the studied taxa.

Results of Statistical Analysis

The anatomical measuments of the investigated species were shown in Table 1. Significance of the differences between the investigated taxa was evaluated by Pearson correlation analysis, One-way ANOVA and Variance analysis. The statistical analysis of the results was given in Tables 2-4. In Table 2, One-way ANOVA test was applied between the anatomical features of P. russeliana and P. armeniaca. According to this table there are meaningful differences between the investigated species at the significance level of 0.05 with P: 0,044* (Table 2). As seen in laboratory studies, P. russeliana and P. armeniaca have similar anatomical features. Thus, this result supports the statistical results (Figures 1-4).

Source	DF	SS	MS	F	P
Factor	11	5296	481	2,82	0,044*
Error	12	2052	171		
Total	23	7348			
S = 13,08 R-Sq = 72,08% R-Sq(adj) = 46,49%					
Individual 95% CIs For Mean Based on					
Pooled StDev					
Level	N	Mean	StDev	------+---------+---------+---------+---	
1	2	19,00	5,66	(-------*-------)	
2	2	31,00	8,49	(-------*-------)	
3	2	13,50	6,36	(-------*-------)	
4	2	11,50	7,78	(-------*-------)	
5	2	17,00	1,41	(-------*-------)	
6	2	33,00	21,21	(-------*-------)	
7	2	5,50	0,71	(-------*-------)	
8	2	63,00	36,77	(-------*-------)	
9	2	24,50	2,12	(-------*-------)	
10	2	18,50	3,54	(-------*-------)	
11	2	26,50	4,95	(-------*-------)	

MS: Mean Square; 1-11: Codes of anatomical features; P: Probability; *: P<.05;
Table 2: Correlation between 12 anatomical features (One-way ANOVA)

In Table 3, person correlation test was applied between the root, stem and leaves anatomical features of the species and significant differences were found between 1-4, 5-6, root and stem features at the significance level of 0.05P and 0.01P. On the other hand, no statistical similarity was observed between the leaf anatomical features of the studied species (Table 3). A similar situation was observed in the root sections of the species. According to figure 1 and 2, Phlomis russeliana differs from the other species with xylem elements filling the center at the root. This situation was also detected in statistical studiest (Table 4). According to the table, there is no statistical similarity in the root xylem properties represented by 4 and has a NS (Not Significant) value.

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	1	2	3	4	5
2	0,263				
	0,737				
3	0,262	0,932			
	0,738	0,068			
4	0,983	0,142	0,099		
	0,017*	0,858	0,901		
5	0,167	0,960	0,990	0,012*	
	0,833	0,040*	0,010**	0,988	
6	0,285	0,982	0,983	0,141	0,990
	0,285	0,018*	0,017*	0,859	0,010**

* Significant at the level of P< 0.05. ** Significant at the level of P< 0.01. Abbreviations: 1,2: root; 3,4: stem; 5,6: leaf ; Codes of anatomical features; (1-3-5): ***Phlomis russeliana***; (2,4,6):***Phlomis armeniaca***

Table 3: Pearson's correlation based on root, stem and leaf anatomical features.

Table 4 shows the relationship of 12 anatomical features of the studied species with each other using the variance analysis test. According to the table, there are statistically significant between especially stem anatomical features of the studied species at level of 0.01P and 0.05P (Table 4). So, according to both statistical methods, it was determined that there are statistically significant between the anatomical features of investigated species. On the other hand looking at the section photos taken from the microscope, it has been observed that the anatomical sections obtained of the studied species have similar anatomical features (Figures 1-4). So the laboratory results on the species confirm the statistical results obtained.

Source	MS	F-value	Probability	Significance
1-2	144.0	2.770	0.238	NS
1-3	30.30	0.830	0.457	NS
1-4	56.30	1.220	0.384	NS
2-7	65.30	17.30	0.050	*
2-12	61.30	16.30	0.050	*
3-7	63.00	31.10	0.219	NS
4-11	169.1	5.310	0.150	NS
5-7	132.2	105.8	0.009	**
5-9	56.20	17.60	0.050	*
5-12	110.0	88.10	0.011	**
6-10	210.1	0.960	0.460	NS
7-9	361.5	144.7	0.007	**
7-10	167.5	25.00	0.036	*
7-12	441.8	35.10	0.027	*
8-10	36.65	4.170	0.148	NS
9-12	324.5	291.6	0.008	**
10-12	144.5	22.39	0.030	*

MS: Mean Square; *P<.05; **P<.01; 1-12: Codes of anatomical features; NS: Not Significant.

Table 4: Correlation between 12 anatomical features (Analysis of Variance)

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In this study, anatomical properties of two endemic Phlomis species that spread in the northern and western Anatolian part of Turkey were examined and the results were evaluated statistically. As a result, when the two species are compared with each other, it has been determined that they mostly show similar anatomical features except for some differences. These similarities and differences have been confirmed by both laboratory and mathematical evaluations. By the analysis of the investigated species 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.

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