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Taxonomic study in *Perovskia* Karel. by checking the morphologic, anatomic and phytochemical aspects in Iran

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Abstract. Genus *Perovskia*, a small and unknown family of Lamiaceaea, grows in the form of herbaceous and shrub. *Perovskia* has been reported in 3 species and 2 hybrids. Due to the lack of baseline data for the taxonomy of this genus, morphology, anatomy and chemotaxonomy of 13 sample populations in Isfahan, Khorasan and Sistan-Baluchistan were evaluated. In morphological Studies, minor differences were observed in 18 traits between populations, of which heterostyly was the most important differentiation. In terms of anatomy of stem no significant differences were observed between populations. The compound of essence in 4 populations was analyzed and 10 main compounds with the highest percentage in populations were similar. Therefore, based on the observations made, in the present study the adoption of two species of *Perovskia* instead of three is recommended. Accepted species are the one with larger stamens than style called *P. abrotanoides* Karel and the one with shorter stamens than the style called *P. atriplicifolia* Benth..

Keywords: Taxonomy, heterostyly, *P. abrotanoides* Karel., *P. atriplicifolia* Benth.

1. INTRODUCTION

Lamiaceae family with approximately 252 genera and 6,800 species is worldwide and because of the production of volatile oil is used in pharmaceutics [1]. *Perovskia* is one of the small but highly valuable spieces in Lamiaceae family, which was described in 1841 by Karelin. *Perovskia* is used in treating leishmaniosis [2], malaria [3], fever [4], soothing rheumatic pains, [5] and controlling infection [6]. 12 names are listed in ipini database for *Perovskia* and 3 species of these, namely *P. abrotanoides* Karel., *P. atriplicifolia* Benth., *P. artemisioides* Boiss and two hybrids namely *P. atriplicifolia* × *P. artemisioides*, *P. atriplicifolia* × *P. abrotanoides* have been reported in Iran. [7,8] There is an agreement on the presence of these three species among experts but the presence of two hybrids with the intermediate morphology cause distortions of their boundaries. However, anatomical and phytochemical aspects may solve the problem. Morphology is one of the most important sources of data in biosystematics, which gives the highest number of taxonomic traits in preparing the classification systems [9] Morphology along with other traits is used in phenetic analysis in the form of data set, as morphological data characterize the genetic condition of plant and can be used along with other traits to study the evolution of plants. Furthermore, the use of anatomical features is more prominent than morphological features as the internal compounds of a plant are less affected in terms of genetic and phenetic than the surface compounds. Today, all aspects of the anatomy of

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plants are studied by professionals in taxonomy [10]. Secretory trichomes are one of the most important traits in Lamiaceae family, which produce essential oil, too [11]. In terms of chemotaxonomy, plants produce different natural products. In many cases, the distribution of natural products and their different ways in biosynthesis help us in taxonomic categorization. The compositions used in chemotaxonomy include three major groups, namely semantids, primary and secondary products. In general, secondary products and semantids are the main chemical compounds that have been shown useful for taxonomy [10]. Therefore, Morphology and chemotaxonomy aspects were investigated to study the taxonomy of the genus *Perovskia*.

2. MATERIALS AND METHODS

13 samples of the genus *Perovskia* were collected from their natural habitat (Sistan-Baluchistan and Khorasan) by Ms. Salimi and Dr. Joharchi, and then the samples were identified in the herbarium of Isfahan University. Also, the plant sheets were kept in herbarium by 16278 voucher specimen number science faculty of Isfahan University.

2.1. Methodology of the morphological studies

To identify the samples, the books Flora Iranica, [7] color flora of Iran [12] and flora of Iran [13] were used. In each of the sample populations, characteristics of the leaves, flowers, stems and petioles (Table 1) were studied. Using a dissecting microscope and graph paper studies were carefully checked.

2.2. Methodology of anatomical studies

Leaves, stems and petioles of each sample were put in the glycerin: alcohol: water (1: 1: 1) fixator solution separately and samples were removed from the fixator after 25 days and rinsed with water. The flowers were boiled for 1-2 minutes instead of fixator solution. Slicing from different parts of the stems, leaves and petioles was done by hand. For cutting by hand, a small piece of stem, leaf and petiole were placed in polystyrene and thin slices of each sample were taken with the use of a sharp blade. After slicing to determine the different parts of the stem, leaf and petiole, samples were colored by double staining using methyl green and Zaji Carmen [14].

2.3. Methodology of phytochemical studies

2.3.1. Methodology of extracting essential oil from flowers

To hydro-distillate, about 24 grams of powdered flowers of species number 1-4 along with 10 times of distilled water (approximately 240 cc) were poured into the flask and was placed in an electric oven. Extraction system installed on the flask and distilled water was added in the pipe so that the amount of it in the three pipes was the same. Vapors of boiling flowers passed the refrigerant and condensed in adjacent to cool water and the essential oil flouted on water. Finally after the completion of boiling (about 5-4 hours) and by opening the valves, the essential oil was poured in small glasses. Then, the combinations of 4 flowers were analyzed by the use of GC-MS device. [15]

3. RESULTS

The data from the study of the morphology and chemotaxonomy of genus *Perovskia* Karel. is as follows:

3.1. Morphological studies

They are shrubs to a height of 50 to 150 cm, with numerous branches. Their stems have short branches. The inflorescence consists of a cycle apart from each other and is in clusters. Flowers

have a short peduncle or they lack it. Siphonocalyx, connected at the top of the rack, in more or less maturity, with 5 ribbed, with three short teeth on the upper edge and more or less integrated, two teeth in the lower edge. Purple corolla in available species in Iran; the length of it is twice the length of corolla; funnel-shaped; with an incomplete ring of trichome, and two edges; the upper edge with four almost equal lobes; the lower edge, integrated and undivided; four stamens, two short and two long stamens; the upper stamen are fertile and attached to the upper edge of corolla (Epipetalus) and sometimes are out of the corolla; anther sacs are parallel to each other and narrow. Two lower stamens are sterile and hidden in corolla. Gynobasic style, crotched stigma, flat, almost like a page with wavy margins. Obavatus achene, round top, a smooth surface with parallel dark lines.

The structure and morphology of trichomes in all organs (stems, petioles and leaf) are the same, and they include two types of coating (non-glandular) and secretory trichome (glandular). Non-glandular trichomes include simple (unicellular to multicellular) and branched types (crotched or multipronged) and glandular trichomes include Capitate and Peltate types (two or more cells) (Figure 1).

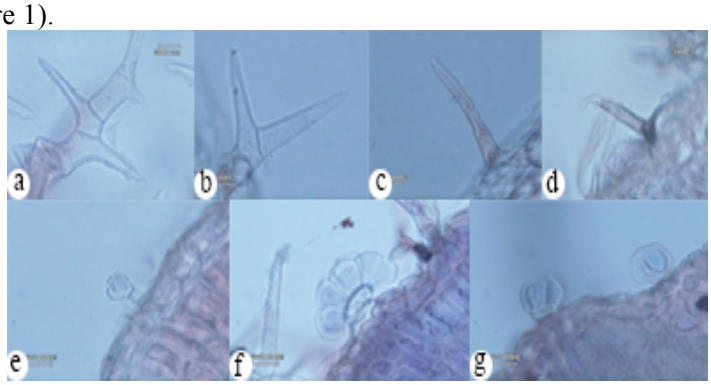

Figure 1. Trichomes morphology in populations of *Perovskia*
a,b. branched nonglandular trichome of population 6 in Sistan and Baluchistan.
c,d. simple nonglandular trichome of population 12 in Isfahan. e. captate glandular trichom of population 4 in khorasan. f. peltate glandular trichom with multicellular head of population 8 in Sistan and Baluchistan. g. peltate glandular trichom with dicellular head of population 2 in Sistan and Baluchistan.

As Table 1 shows, 18 morphological traits in the sample populations were studied which differed in detail.

Table 1. Morphological study in populations of *Perovskia.*

	Morphological traits	
1	Inflorescence	Racemose
2	Leaf apex	Obtuse
3	Sepal number	5
4	The Calyx ribs number	5
5	The sepals toothing number	3+2
6	Corolla form	funnel-shaped
7	Petal number	5
8	The corolla clefts number	2
9	The corolla lips number	1+4
10	Petal apex	Roundish
11	Stamen number	4(2+2)
12	Stamen type	Epipetal
13	Anther form	Narrow and parallel
14	Location of anthers	In two sides of lips
15	Style form	Bifurcate and flattened

16	Achene form	Obovate
17	Achene surface	With parallel dark lines
18	Trichomes structure	All the same

3.2. Anatomical studies

3.2.1. Stem overview

Stem consists of three tissue systems including epiderm, coretx and vascular system. epiderm tissues are made of an layer with rectangular cells. In stem, cortex tissue differ with cortex and pith. cotrex is often parenchyma and consists of spherical or oval cells with thin walls and a lot of space between cells. The outer layers of cortex cells are involved in photosynthesis due to having chloroplast. The innermost layer of the cortex is called the endoderm which sometimes consists of cells with thickening of Caspar bar. The most important component of the vascular system is the stele which consists of vascular bundles (Figure 2).

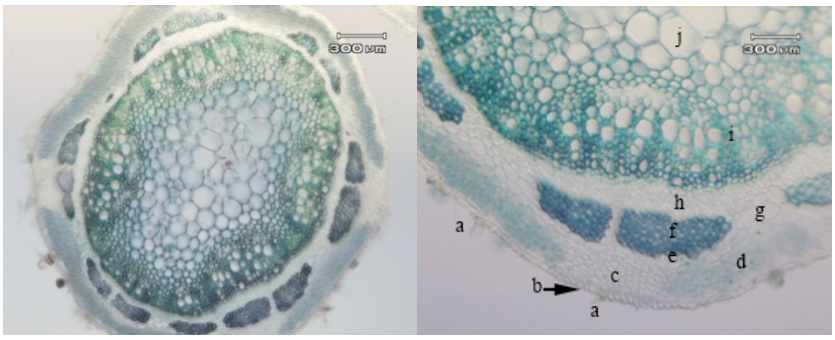

Figure2. Stem transection of population 4 in khorasan.
A.glandular and nonglandular trichomes b.epiderm c.hypoderm(colanshym) d. cortex (paranshym) e.andoderm f.scloranshyma fibr g.paranshym h.pheloem i.xylem j.pith.

3.3. Chemo-taxonomical studies

3.3.1. Essential oil

The essence of flowers of 4 sample populations (populations number 1, 2 and 3 from Sistan-Baluchestan and 4 from Khorasan Province) was extracted and the compounds identified are presented in Table 2.

Table 2. Essential oil of 4 populations in Khorasan and Sistan and Baluchistan.

Compounds	%(1)	%(2)	%(3)	%(4)
Tricyclene	-	0.19	0.22	
α-Pinene	9.98	8.18	6.97	8.40
Camphene	2.86	2.84	2.64	3.91
Isopropyl tiglate	-	-	0.22	
β-Pinene	1.37	0.15	1.6	1.87
Myrcene	0.57	0.72	0.74	0.87
α-Phellandrene	-	0.29	0.29	-
δ-3-Carene	11.23	9.76	8.63	6.13
α-Terpinene	11.23	0.54	0.61	0.37
Para-Cymen	0.87	-		0.78
1,8-cineole	18.65	15.84	14.41	15.52

Ocimene	-	0.17	0.82	0.35
γ-Terpinene	0.68	0.88	0.98	0.63
α-Terpinolene	3.60	2.96	3.52	0.47
Camphor	2.64	2.08	1.97	19.22
Borneol	5.60	5.74	6.18	5.60
α-Terpineol	0.57	-	-	-
γ-Terpineol	2.07	-		1.10
Terpinene-4ol.	-	5.57	0.65	0.47
Sabinene hydrate acetate	-	2.09		
Geraniol	-	2.39	1.96	
Para-Cymen-8ol.	-	-	0.20	
Fenchol<2ethyl endo>	1.81	-	2.33	
Linalyl oxide acetate	0.95	-	1.96	
Linalyl acetate	1.62	-	-	
Bornyl acetate	2.69	3.15	3.01	2.43
Terpinenyl acetate	-	-	1.95	2.19
α-Copaene	-	-		0.48
α-Gurjunene	-	-	-	0.92
Limonene aldehyde	-	1.92		
Neryl acetate	6.63	5.55	3.39	
β-Caryophyllen	7.19	6.21	8.18	5.62
α-Humulene	7.55	6.51	8.33	4.85
α-Patchoulene	-	0.24	-	
Alloaromadendrene	-	-	0.24	
Dehydroaromadendrene	-	-	0.30	
α-Amorphene	-	0.45	0.54	
-Epizonaren	-	-		0.54
γ- Cadinen	-	--		2.69
δ- Cadinen	-	0.29	0.39	1.69
Valencene	8.45	-	-	
Caryophyllene oxide		0.55	0.60	0.61
Viridiflora		8.10	7.88	
Leden	1.25	2.00		
Humulene epoxide			0.72	
Naphthalene-2acetyl			0.42	1.36
Epi-α-Cadinol			1.91	7.03
β-Eudesmol		0.49	0.50	
α-Eudesmol		0.95	0.93	0.49
Caryophyllene		0.34		
Bisabolen	0.95	1.03		0.50
Valeranone		0.42	0.43	
Farnesyl acetate		1.44		
α-Santalol			1.72	

3.4. Identification and naming of investigated populations of Perovskia

The key to identify *Perovskia* Karel. Species based on Iranian flora and flora Iranica is as follows:

1. Simple, undivided leaves, toothed on the margin…..……..... *P. atriplecifolia*

-Pinnatiscect leaf……..…… 2

2. The stem and leaves are covered with dense stellate trichomes..... *..P. artemisoides*

-leaves are without trichomes. Fewer stellate trichomes and caduceus... *P. abrotanoides*

4. DISCUSSION

Based on morphological, anatomical and phytochemical observations made and based on the discussions below, the integration of *P. atriplecifolia* and *P. artemisioides* species and adoption of two species instead of three for Iran's flora is recommended.

4.1. Discussions on the integration of Species

4.1.1. Chromosome number

Based on the available data in IPCN database, chromosome number of three species, *P. abrotanoides* Karel., *P. atriplicifolia* Benth., and *P. scrophulariifolia* Bunge, have been reported as n = 10 and 2n = 20.

4.1.2. Morphology

After investigating 18 morphological traits no clear distinctive traits among *Perovskia* species were observed (Table 1). Despite the emphasis on trichomes in *Perovskia*, the structure and morphology of them in different organs (stems, petioles and leaf) are the same and they consist of glandular trichomes (basic and non-basic) and non-glandular trichomes (simple or branched).

4.1.3. Leaf morphology

Texture and morphology of leaves in *Perovskia* species ranged from simple to deep cutting and there are no clear boundaries (figure 3). Therefore, defining them as species is hardly possible.

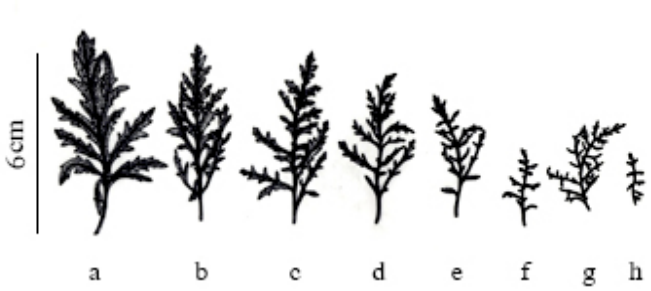

Figure3. Rang from simple to partite leaves in populations of *Perovskia.*
a. population 1 from Sistan and Baluchistan. b. population 5 from Sistan and Baluchistan. c. population 8 from Sistan and Baluchistan. d. population 2 from Sistan and Baluchistan. e. populations 3, 9, 10, 12 from Sistan and Baluchistan. f. populations 6, 7 from Sistan and Baluchistan. g. population 4 from Khorasan. h. populations 6, 7 from Isfahan.

4.1.4. Anatomical structure

Stem includes skin tissues, underling tissues and the vascular system. The overall structure of the stem in 13 sample populations in Isfahan, Khorasan and Sistan -Baluchistan was similar.

4.1.5. hybridization

Due to the number of reports concerning the hybridization of *Perovskia*, hetrostyly is briefly explained, which is the main factor both in hybridization and preservation of boundaries of taxons. Hetrosyly is a genetic polymorphism which occurs in some species due to differences in the size of style and includes distyly and tristyly (Figure 4).

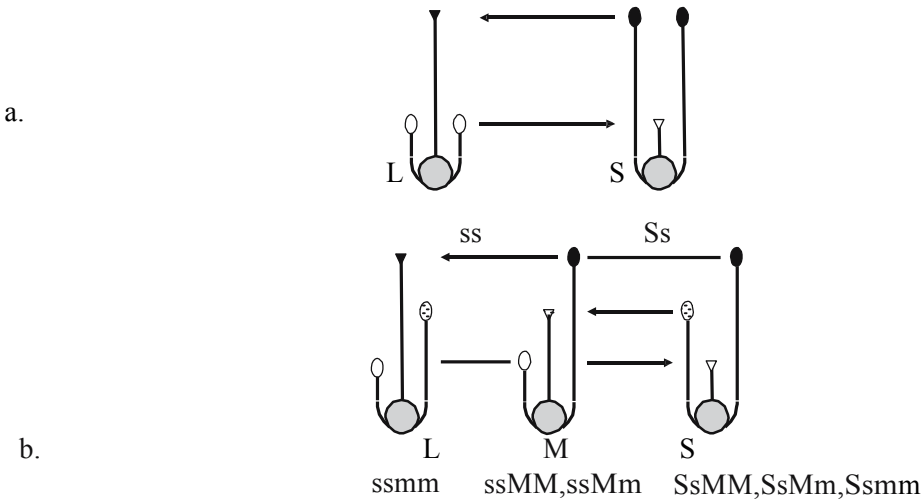

Figure 4. Heterostyly. a. Distyly. b. Tristyly. from (Barret, 1990) [17].

Heterostyly trait can be seen in at least 28 plant families, including («Hugonia» Linaceae, «Primula» Primulaceae and «Lythrum» Lythraceae). The presence of flags above the style increases cross-fertilization which may be improved further in case of self-incompatibility between pollen and stigma. In this case, the number of unused pollen decreases. In the case of lack of self-incompatibility, we can observe increased self-fertilization which leads to self-pollinated populations in large numbers (Barret, 1990).

Accordingly, in 10 out of 13 sample populations under the study style was longer than stamens (distyly). This attribute can cause kind of self-incompatibility in populations (3 populations with larger style than stamens). As a result, this provides the possibility of hybridization between populations and it gradually reduces the number of plants with style larger than stamens.

4.1.6. Ecology

Ecologically, *Perovskia* species are limited to northeastern, central and eastern regions (Jamzad, 1391) and cold and dry mountainous areas in Iran. So far there are no reports concerning the Zagros Mountains.

4.1.7. Essence compounds

According to the results with regard to extracting essence obtained from 4 sample populations (Table 2) the compounds had low diversity and 10 major compounds (with highest percentage) in all 4 populations were similar (Table 3).

Table 3. Comparison of essential oil in 4 population Based Purification of Table 2.

compounds	Composition of flower in the population 1	Composition of flower in the population 2	Composition of flower in the population 3	Composition of flower in the population 4
1,8-cineole	18.6	15.84	15.52	14.41
δ-3-Carene	11.23	9.76	6.13	8.63
α-Pinene	9.98	8.18	8.40	6.97
β-Caryophyllen	7.19	6.21	5.62	8.18
α-Humulene	7.55	6.51	4.85	8.33
Borneol	5.60	5.74	5.60	6.18
Camphor	2.64	2.08	19.22	1.97
Viridiflora	8.45	8.10	Trace	7.88
Neryl acetate	6.63	5.55	Trace	3.91
Epi-α-Cadinol	Trace	Trace	7.03	1.91

According to the observations made and discussions above and based on differential characteristics of heterostyly, two species of genus *Perovskia* is accepted as follows:

1- Stamen longer than style…….……..…….. *P. abrotanoide*

2- Stamen shorter than style……...…….. *P. atriplicifolia*

1.*P. abrotanoides* Karel, in Bull. Soc. Nat. Mosc. 14: 15 (1841).

Shrubs or bushes to a height of 1.5 m; Stems covered with stellate and glandular secretory trichomes; Leaves with petiole, about 4 to 7 cm in length; Blades, about 5.4 to 10 cm in length; bipectinate, almost glabrous; leaves in inflorescence are similar to leaves in stem, but smaller; Cluster inflorescence; Bracteole, 5.1 to 2.5 mm in length, spoon-like, bayonet-like; Corolla, about 3 to 6 mm in length, tubular; teeth in pricky top are covered with simple multicellular trichomes with purple bands and secretory glands without with or orange pedicel; upper edge with three almost attached teeth; smaller middle teeth and lower edge with two short teeth; Corolla tube is out and flags are out of the corolla; achene , 7/1 to 7/2 mm in length, obovate; flat surface with parallel lines; habitat in Iran: Northeastern, eastern and central regions.

2.*P. atriplicifolia* Benth. in DC., 12: 261 (1848).

Straight-stemmed shrub; stems covered with stellate and glandular secretory trichomes; Petiole, 2 to 10 mm in length; Blade 2.5 to 4 cm in length and covered with dense crotched trichomes with white or yellow glands; A Cluster inflorescence; Bracteole 5.1 to 2.5 mm in length; Corolla, 3 to 5.5 mm in lenght, covered with stellate and glandular secretory trichomes without yellow pedicel. Corolla 5 to 11 mm in lenght; upper edge lobes in corolla have dark purple stains; Flags are hidden inside the corolla; achene was not observed; Habitat in Iran: eastern regions.

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