

## HISTO-ANATOMICAL AND BIOCHEMICAL ASPECTS REGARDING AERIAN VEGETATIVE ORGANS OF *PRUNELLA VULGARIS* L.

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**Abstract.** The *Lamiaceae* family includes many aromatic and medicinal plants, with a high economic importance. Many species of this family possess volatile oils secreted by glandular hair located on their aerial vegetative organs and some of their reproductive organs. In this paper were analyzed from histo-anatomical point of view, the aerial vegetative organs of *Prunella vulgaris*, species largely spread in our country. The essential oil was extracted by hydrodistillation, the separation and identifying of their components were made using the gas-chromatograph coupled with masspectometry.

**Key words:** *Lamiaceae*, glandular hair, volatile oil

### Introduction

The genus *Prunella* (*Lamiaceae*) consists of 9 species, 2 subspecies and 1 variety in the world and 4 species and 2 subspecies in Europe [2]. In Romania, *Prunella* genus is represented by 3 species: *P. vulgaris* L., *P. grandiflora* L. and *P. laciniata* L. [5].

*P. vulgaris*, known as common selfheal, heal-all, heart-of-the-earth, is a common medicinal plant in our country, being often spread in grazings, glades, orchards or on lakesides. The plant presents a repent or oblique rhizome in soil, the stems are ascendants, 40-50 cm height, glabres or with few hairs. The leaves are lance shaped, serrated with long petiole, with the mention that the ones from the base of inflorescence are sessile or present a short petiol [16].

The plant can be used in salads, soups, stews or boiled as a pot herb. Also, it has been used as an alternative medicine for centuries on just about every continent. The infusions of *P. vulgaris* flowers were used against the mouth and thorax ulcers as gargle in Germany. While the inflorescens of *P. vulgaris* have been utilized as expectorant, the aerial parts of this plant have been used against hemorrhage, hemorrhoid, diarrhea and dysentery. Furthermore, the infusions of *P. vulgaris* were mixed with honey and used as tonic in Europe [8]. In India and China, *P. vulgaris* has been used against pulmonary disease, jaundice, liver inflammations and as antipyretic. Moreover, it has been used as laxative, anticough, antiparasitic, antirheumatic, against vertigo and hemorrhoid as well as for eye and ear diseases in India [18]. In Ozbekistan, *P. vulgaris* is recorded as a plant which is useful in cardiovascular diseases [15]. Furthermore, *P. vulgaris* flowers have been reported as diuretic in Japan [11]. In Turkey, the aerial parts of *P. vulgaris* have been used as antirheumatic, antiarrhythmic and against common colds [21].

### Material and methods

The aerial parts of *P. vulgaris* L. were collected during the flowering period from Iași district. For the histo-anatomical research, the vegetal material has been fixed and

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preserved in 70% ethylic alcohol. The sections were cut with a microtome and a botanical razor. The aerial vegetative organs were cross sectioned, on different levels, from the top to the basis. First of all, the sections were submitted to a discoloration process, using sodium hypochlorite (20-25'), then colored (with iodine green and with ruthenium) and mounted in gel. The drawings were performed by means of a Romanian (Projektionszeichenspiegel) microscope and the micrographs were performed by means of a Novex (Holland) microscope, using a Canon A95 camera.

The volatile oils have been extracted using a Clevenger type apparatus according to the European Pharmacopoeia standards, in the Laboratory of Plant Physiology, Faculty of Biology, "Alexandru Ioan Cuza" University of Iași. The separation and the identification of the components have been carried out using GC-MS (gas chromatography coupled with mass spectrometry) at the Faculty of Horticulture, U.S.A.M.V. București.

## **Results and discussions**

### **Histo-anatomical aspects**

#### **The stem (Plate I, fig. 1-6)**

In cross section of the stem the epidermis cells are isodiametrical or slowly tangentially prolonged, having the external wall thickened, but covered by an extremely thin cuticle. Here and there, tector and secretory trichomes are present, especially in the ribs. The tectory trichomes are more frequently in the middle and inferior level of the stem, being mostly multicellulars than unicellulars.

The glandular trichomes are always multicellular having a basal cell, smaller than the epidemical ones between which it is situated, unicellular short stalk and a gland made of 1 or 2 cells, covered with a common, curved cuticle. Still of the epidemical level there are stomates, whose cells are little curved over the level of the protective cells. In the inferior third of the stem we notice that the frequency of the glandular trichomes is reduced.

The cortex is relatively thin, parenchymatous assimilator, of meatic type between the ribs and predominantly of colenchymatous type inside the ribs. The cortex ends in a casparyan type of endoderma, having tangentially prolonged cells of different size.

The central cylinder presents a secondary structure (except the superior level) resulting from the activity of the cambium: a very thin ring of phloem and a thick ring of xylem. Phloem cells forms a thin ring at the external part of central cylinder (consisting in sieved tubes, companion cells and less parenchyma cells) and xylem cells forms a ring at its internal part, with vessels irregular dispersed, separated by libriform fibers, which bear strong sclerified and intense lignified walls.

The pith is thick, parenchymatic-cellulosed, of meatus type.

#### **The foliar limb (Plate II, fig. 7-12)**

In front side view, the epidermis consists in elongated cells, with sinuous lateral walls. Epidemical cells are irregular, rounded or tetragonal in shape. A few tector trichomes, of different size, are present at the edges of the limb; their structure varies from unicellular to pluricellular. Here and there secretory trichomes (always multicellular having a basal cell, a unicellular short stalk and a gland made of 1 or 2 cells) are present. Both epidermis present stomata of diacytic type, so, the limb are amfistomatic.

The vascular bundles are big and present a very thick sclerenchymatic sheath at the phloemic pole. The mesophyll is formed by palisade tissue and spongy tissue; the

former is bi-layered, but the cells belonging to the hypodermic layer are higher than the others.

General anatomical information of *Lamiaceae* and some special anatomical features of stem and leaves of *Prunella* L. genus have been previously reported by Metcalf and Chalk. In 1972 the anatomical study of stems and leaves of *P. vulgaris* and *P. laciniata* growing in middle Europe has been carried out by Natherova and Rezacova. Recently, Jehad A.H. and Nurten E. (2008) have analyzed the anatomical features of genus *Prunella* L. growing in Turkey.

### **Chemical composition of volatile oil (Plate III)**

Aromatic plants have been used since ancient times for their medicinal properties. These properties can be partially or entirely attributed to their volatile oils that are volatile fraction of aromatic and medicinal plants after extraction by steam or water distillation. They have been used for their pharmaceutical potential since early times, and even now are still subject to a great deal of attention, as is clear from the increasing number of publications each year on this subject [7].

GC-MS analyses of *P. vulgaris* essential oil resulted in the identification of 30 compounds. The main constituent is: germacrene D (48,18%). In smaller quantities  $\alpha$ -pinene (4,54 %),  $\beta$ -elemene (4,12%) and  $\beta$ -caryophyllene (4,45%). All components are listed in Table 1, in order of their elution.

Germacrene D (the main constituent of volatile oil of *P. vulgaris*) is an important sesquiterpene widely occurring in nature and is considered as important intermediates in the biosynthesis of other sesquiterpenes [1]. Germacrene sesquiterpenes have been reported to have various biological purposes and effects in nature [6]. These purposes range from biosynthetic intermediates of several sesquiterpenes [3] and to specific biological activities such as pheromone, anti-cancer agents, antibacterial and antifungal activities [1].

$\alpha$ -Pinene is an organic compound of the terpene class, one of two isomers of pinene [19] with important biological activities (antibacterial, antispasmodic, expectorant, fungicide, herbicide, pesticide) [23].

$\beta$ -Elemene has been used as an antitumor drug for different tumors [22].

The plant secondary metabolite,  $\beta$ -caryophyllene, is a natural bicyclic sesquiterpene of many plants that has traditionally been used in the cosmetics industry to provide a woody, spicy aroma to cosmetics and perfumes. Clinical studies have shown it to be potentially effective as an antibiotic, anesthetic and anti-inflammatory agent [17].

### **Conclusion**

Our results regarding the histo-anatomical researches confirm the existent dates from the literature, very few and with a general character [2], [13] and, on the other hand bring new information. Concerning the volatile oil composition, we have obtained 30 compounds, the main constituent being germacrene-D.

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### Explanation of the plates:

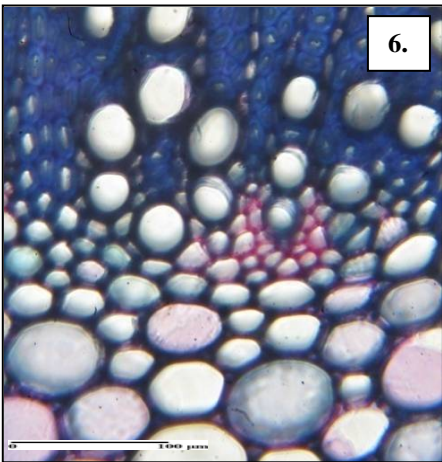
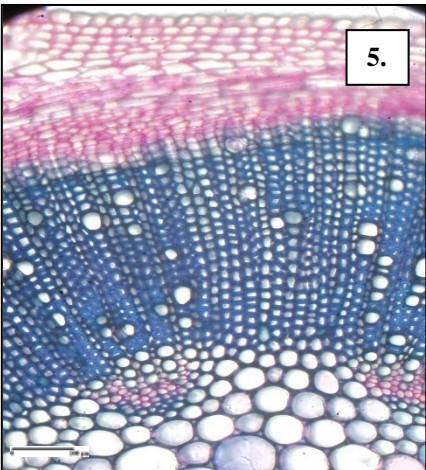
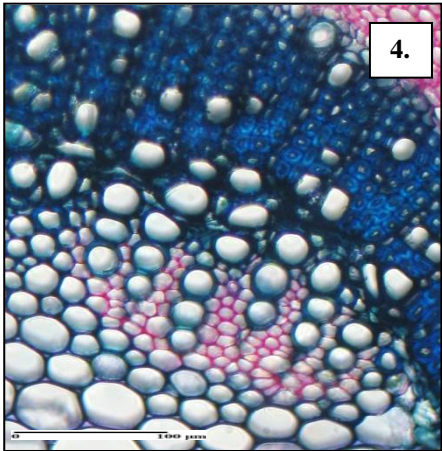
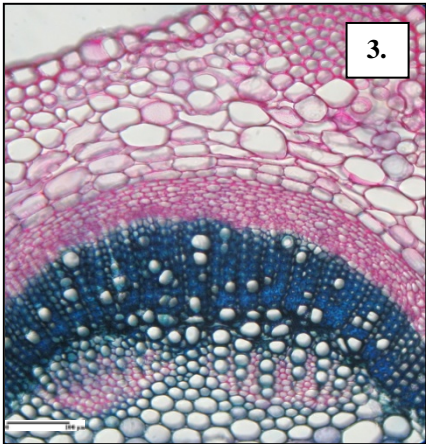
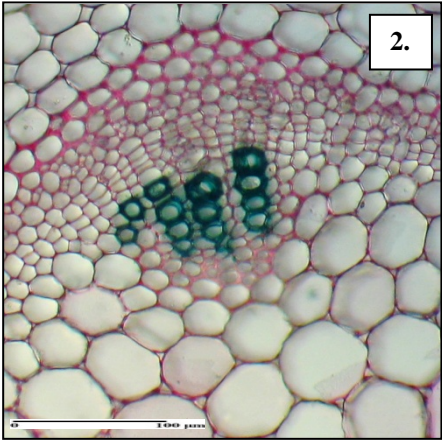
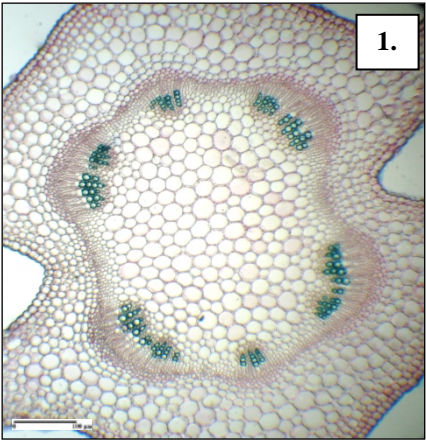
#### **Plate I: Cross section through the stem of *Prunella vulgaris* L.**

1. Cross section through the superior level of the stem
2. Cross section through the superior level of the stem – detail
3. Cross section through the middle level of the stem
4. Cross section through the middle level of the stem – detail
5. Cross section through the inferior level of the stem
6. Cross section through the inferior level of the stem – detail

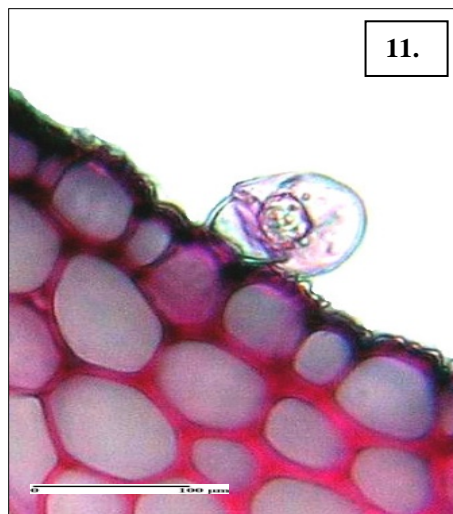
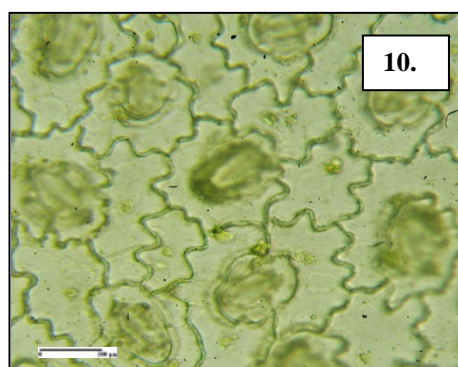
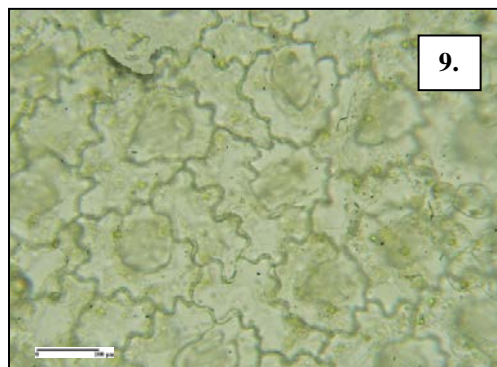
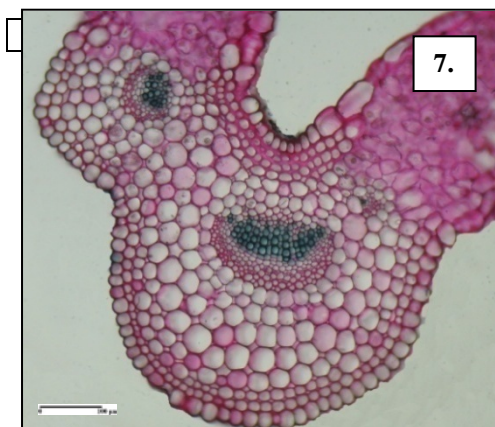
#### **Plate II: Cross section through the foliar limb of *Prunella vulgaris* L.**

1. Cross section through the limb
2. Cross section through the limb – detail
3. Glandular trichome with a unicellular gland
4. Glandular trichome with a bicellular gland

#### **Plate III: Chemical composition of volatile oil of *Prunella vulgaris* L. (table and graphic)**







1.	$\alpha$ -tujen	0,59	16.	bornyl acetate	2,45
2.	$\alpha$ -pinene	4,54	17.	$\alpha$ -terpinyl acetate	0,21
3.	camphene	1,99	18.	$\beta$ -burbonene	1,99
4.	$\beta$ -pinene	2,28	19.	$\beta$ -elemene	4,12
5.	pentamethyl heptane	1,10	20.	$\beta$ -caryophyllene	4,45
6.	terpinolene	0,26	21.	$\beta$ -cubebene	0,47
7.	cymol	2,44	22.	epiglobulol	0,48
8.	limonene	3,22	23.	$\alpha$ -cariophyllene	1,15
9.	cyneol	3,65	24.	ermurolen	0,38
10.	$\beta$ -trans-ocimene	0,45	25.	<b>germacrene D</b>	<b>48,18</b>
11.	gamma-terpinene	2,77	26.	$\alpha$ -farnesene	0,86
12.	linalool	0,55	27.	elisen	3,73
13.	p-menthone	0,69	28.	farnesene	0,51
14.	borneol	0,76	29.	$\beta$ -cadinene	0,83
15.	carvene	1,30	30.	caryophyllen oxid	0,45

