



## CHARACTERISTIC OF LEAF PELTATE GLANDULAR TRICHOMES AND THEIR VARIABILITY OF SOME LAMIACEAE MARTINOV FAMILY SPECIES

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Glandular trichomes are important taxonomic characters in the Lamiaceae family. The objects of our investigations were plants of Lamiaceae family collected from the experimental base of Institute of Rice of the National Academy of Agrarian Sciences (Plodove, Ukraine). Fresh leaves were collected for the morphological analyse. The dimensions of glands (diameter in  $\mu\text{m}$ ) were measured using of microscope Discovery V12 Carl Zeiss at the Institute of Biodiversity Conservation and Biosafety of the Slovak University of Agriculture in Nitra (Slovakia). Peltate glandular trichomes with essential oil are localized on the adaxial and abaxial leaf surfaces. Their size on the adaxial and abaxial leaf sides is different. The diameter of trichomes on the adaxial and abaxial side of leaves was 65.70 and 61.41 (*Nepeta transcaucasica* Grossh.), 70.10 and 74.54 (*Salvia officinalis* L.), 72.02 and 67.39 (*Monarda fistulosa* L.), 80.53 and 85.11 (*Ocimum basilicum* L.), 94.35 and 105.12 (*Satureja montana* L.), 95.11 and 60.37 (*Thymus serpyllum* L.), 96.48 and 90.57 (*Thymus vulgaris* L.), 106.88 and 107.69 (*Hyssopus officinalis* L.), 110.61 and 88.22 (*Hyssopus angustifolius* Bieb.), respectively. It was noted that the leaf adaxial side of *Salvia sclarea* L. had no glandular trichomes of lamiaceous type at all but on an abaxial side of leaves only with the diameter 96.47  $\mu\text{m}$ . The tested plant species showed significant differences in size and colour of peltate glandular trichomes producing essential oils. This difference can be used during macroscopic analysis of herbal substances. Aromatic plants that were grown in Steppe zone of South Ukraine are perspective plants because of presence a well-generated excretory system which contains essential oils.

**Keywords:** Lamiaceae, leaf, glandular trichome, morphometric characteristic

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## Introduction

Medicinal herbs producing essential oils are economically important for food, pharmacy and cosmetics industry (Mashanov et al., 1988, Shanaida et al., 2018). Therefore, there are many teams oriented on the experimental study of these species (Nesterenko et al., 1937; Muhortova et al., 1973; Simonov et al., 1987; Hlypenko et al., 1987; Rabotyagov and Korsakova, 2000; Kovtun-Vodyanytska, 2017). Species investigation is often focused on cultivation environment and plant ontogenesis effects on physiological/biochemical processes leading to synthesis and production of essential oils (Korsakova, 1998; Mishurova, 1991; Scharapov et al. 1987; Svidenko a Rabotyagov, 2000). Many medicinal plants differ in amounts of essential oil produced by stalks, leaves, inflorescences and in structure, localization and density of glandular trichomes. Inflorescences and leaves produce the highest amount of ether oils (Svidenko, 1999). It is generally accepted that essential oils are formed in unicellular or multicellular glandular trichomes (peltate and capitate ones) of some members of Lamiaceae Martinov (Baran et al., 2010). According to the European Pharmacopeia, peltate glandular trichomes in members of the Lamiaceae family are also named as secretory trichomes of lamiaceous type or glandular trichomes of the lamiaceous type consisting of epidermal cells, a short stalk cell and a multicellular head (European pharmacopoeia, 2016). In the Lamiaceae family, the morphology, distribution and frequency of glandular trichomes are employed as discriminative characters at subfamily level (Baran et al., 2010, Atalay et al., 2016,). Most of the aromatic genera are in the subfamily Nepetoideae (Baran et al., 2010). As a rule, peltate glandular trichomes have a small, rounded, unicellular stalk and a globular or ovoid head composed of 4, 8 or 12 radiating cells with a raised common cuticle (Baran et al., 2010; Kahraman, 2010; European pharmacopoeia, 2016). Sometimes, a head can even contain 4–8 or 14–18 cells (Marin et al., 2012; Atalay et al., 2016; Shanaida, 2016). A large secretory head usually comprises 1, 2, 4 central cells and 4, 6, 8–10 or 6–14 peripheral cells arranged in one-two concentric circles (Baran et al., 2010; Kahraman, 2010; Atalay et al., 2016). There is a confirmation of the relation between the number and size of glands and amount of produced oils by given plant part. Currently, it is proved that the synthesis and accumulation of essential oils basically take place in glandular cells (Malankina, 2007). There are number of studies on trichome morphology of the Lamiaceae family (Serrato-Valenti et al., 1997; Corsi and Bottega, 1999; Kaya et al., 2003; Krstic et al., 2006; Kamatou et al., 2007; Bagherpour et al., 2010; Kahraman et al., 2010; Seyedi and Salmaki, 2015; Atalay et al., 2016), which confirmed, that the presence of trichomes, their structure and localization character are diagnostic features of taxons and have a significant value in pharmacognosy practice (Shanaida, 2016). Kondratenko (1975) determined the direct correlation between the specialized glands number and the essential oils production in the experiments with the species *Thymus vulgaris* L. adaptability and mutation variability. Based on breeding activities, Rabotyagov (1983) elaborated a model of lavender productivity, documenting the dependence of essential oils production by plant on the number of flowers and amount of glands on plant parts. In spite of the above-mentioned information, there is still a lack of the knowledge of inter-species and intra-species differences as well as the numbers and size of plant glands in some species of the Lamiaceae family. Hence, our experimental activities were oriented on variability study of glandular trichomes dimensions of the lamiaceous type some species of Lamiaceae family.

## Material and methodology

### Locating plants and data collection

Plant material collected from the experimental base of Institute of Rice of the National Academy of Agrarian Sciences (Plodove, Ukraine) and fresh leaves were collected for the morphological analyses. Microscopic study of localization glandular trichomes producing essential oil was conducted at the Institute of Biodiversity Conservation and Biosafety at Slovak University of Agriculture in Nitra (Slovakia). The size of glands occurring on leaves was experimentally evaluated for 10 selected species: *Thymus vulgaris* L., *Thymus serpyllum* L., *Hyssopus angustifolius* Bieb., *Hyssopus officinalis* L., *Satureja montana* L., *Salvia officinalis* L., *Salvia sclarea* L., *Monarda fistulosa* L., *Ocimum basilicum* L., and *Nepeta transcaucasica* Grossh. Plant samples were collected between 2015–2016.

### Morphometric characters

The dimensions of glands (diameter in  $\mu\text{m}$ ) were measured on leaves using of microscope Discovery V12 Carl Zeiss. 5 plant leaves were investigated from every species. 25 measuring were conducted on every side of the leaf. Summarizing whole study, 1250 measurements were conducted.

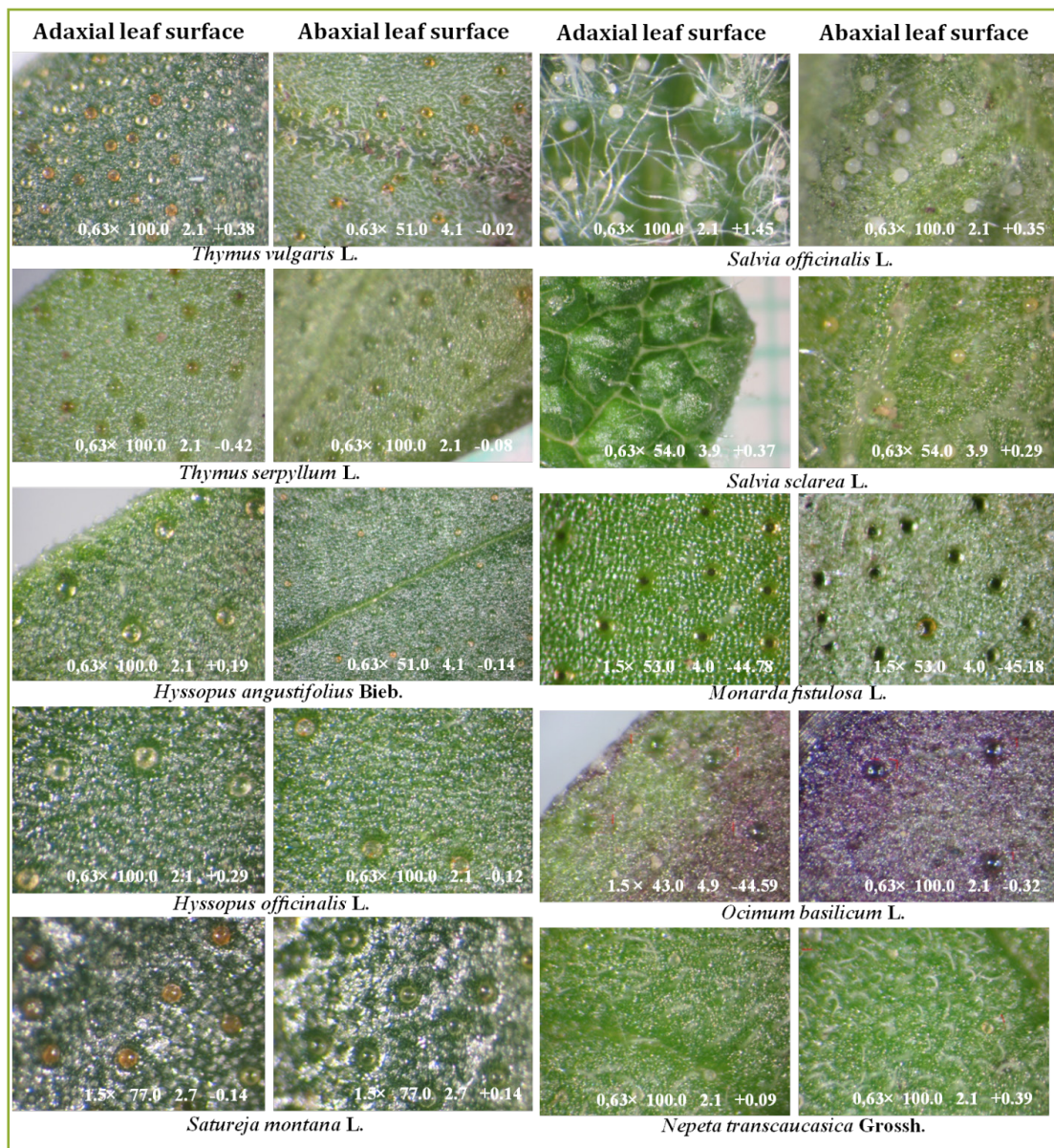
### Statistical analyses

Basic statistical analyses were performed using PAST 2.17; hierarchical cluster analyses of similarity between species were computed on the basis of the Bray-Curtis similarity index.

## Results and discussion

Glandular trichomes are important taxonomic characters in the Lamiaceae family (Xiang et al., 2010). Significant differences were detected in numbers, shape and colour of peltate glandular trichomes (Figure 1). The colour of these trichomes of different plant species varied from transparent to light-yellow or from transparent to red-brown, which depends on the stage of trichome growth. The colour of secretory trichomes of lamiaceous type could be used as an additional tool at carrying out macroscopic pharmacognostic analysis.

Our study confirmed that size and distribution of peltate glandular trichomes have valuable taxonomical significance at the species level (Xiang et al., 2010; Marin et al., 2012; Atalay et al., 2016). Our study established that the size of peltate glandular trichomes depends on species of plants that conforms with conclusions of Baran et al. (2010). Peltate glandular trichomes with essential oil are localized on the adaxial and abaxial leaf surfaces. Their size on the adaxial and abaxial leaf sides is not the same. These trichomes were found of minimum and maximum dimensions from 55.30 (*Nepeta transcaucasica*) to 91.45  $\mu\text{m}$  (*Hyssopus officinalis*) and from 80.19 (*Monarda fistulosa*) to 142.89  $\mu\text{m}$  (*Thymus serpyllum*), respectively (Table 1). On the abaxial side of leaves, peltate glandular trichomes were found and had minimum dimensions from 45.36 (*Nepeta transcaucasica*) to 93.19  $\mu\text{m}$  (*Hyssopus officinalis*) and maximum dimensions from 72.74 (*Thymus serpyllum*) to 123.08  $\mu\text{m}$  (*Hyssopus officinalis*).



**Figure 1** Size, shape and colour of peltate glandular trichome on the adaxial and abaxial leaf surfaces for the selected species of Lamiaceae Martinov family

**Table 1** Variability of the peltate glandular trichome average size on the adaxial and abaxial leaf surface for selected species of Lamiaceae Martinov ( $\mu\text{m}$ )

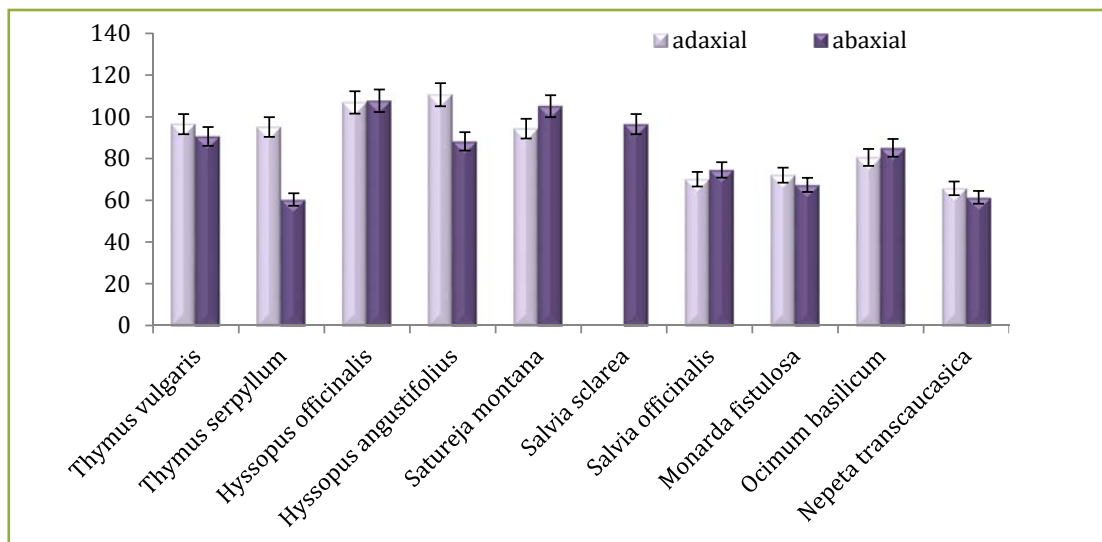
| Plant species                        | Leaf side | min          | max    | Sx    | V%    |
|--------------------------------------|-----------|--------------|--------|-------|-------|
| <i>Thymus vulgaris</i> L.            | adaxial   | 85.61        | 113.02 | 7.47  | 7.74  |
|                                      | abaxial   | 77.96        | 105.97 | 8.08  | 8.92  |
| <i>Thymus serpyllum</i> L.           | adaxial   | 59.13        | 142.89 | 28.49 | 29.95 |
|                                      | abaxial   | 49.96        | 72.74  | 5.65  | 9.35  |
| <i>Hyssopus officinalis</i> L.       | adaxial   | 91.45        | 173.9  | 16.71 | 15.64 |
|                                      | abaxial   | 93.19        | 123.08 | 8.41  | 7.81  |
| <i>Hyssopus angustifolius</i> Bieb.  | adaxial   | 90.09        | 133.89 | 13.44 | 12.15 |
|                                      | abaxial   | 78.72        | 97.43  | 4.40  | 4.99  |
| <i>Satureja montana</i> L.           | adaxial   | 79.91        | 109.09 | 7.56  | 8.01  |
|                                      | abaxial   | 73.67        | 120.04 | 11.07 | 10.53 |
| <i>Salvia sclarea</i> L.             | adaxial   | not detected |        |       |       |
|                                      | abaxial   | 80.93        | 107.83 | 6.98  | 7.23  |
| <i>Salvia officinalis</i> L.         | adaxial   | 60.33        | 85.21  | 6.09  | 8.69  |
|                                      | abaxial   | 65.19        | 83.95  | 4.83  | 6.48  |
| <i>Monarda fistulosa</i> L.          | adaxial   | 64.55        | 80.19  | 4.41  | 6.13  |
|                                      | abaxial   | 58.61        | 77.63  | 5.88  | 8.73  |
| <i>Ocimum basilicum</i> L.           | adaxial   | 68.79        | 92.96  | 7.25  | 9.00  |
|                                      | abaxial   | 67.70        | 99.09  | 8.66  | 10.18 |
| <i>Nepeta transcaucasica</i> Grossh. | adaxial   | 55.30        | 103.25 | 10.53 | 16.02 |
|                                      | abaxial   | 45.36        | 81.48  | 6.77  | 11.03 |

Note: min, max – minimal and maximal measured values; Sx – standard deviation; V – coefficient of variation (%)

According to average values, the least trichomes on the adaxial and abaxial sides of the leaves were found for *Nepeta transcaucasica* – 65.70 and 61.41  $\mu\text{m}$ , respectively (Figure 2).

The biggest size of peltate glandular trichomes on the adaxial side of the leaves was found for *Hyssopus angustifolius* (110.61  $\mu\text{m}$ ) and on the abaxial side of the leaves of *Hyssopus officinalis* (107.69  $\mu\text{m}$ ). Seven species formed larger glands on the adaxial side of the leaves compared to the abaxial ones. Larger peltate glandular trichomes on the upper side of leaves are characteristic for the species *Satureja montana*, *Ocimum basilicum* and *Salvia officinalis*. *Hyssopus officinalis* formed uniformly large glands on both adaxial and abaxial sides of the leaf.

The large peltate glandular trichomes are located in deepenings on the adaxial and abaxial leaf surfaces and are densely distributed on the adaxial and abaxial leaves surface of *Satureja montana* that is in line with studies of Marin et al. (2012).



**Figure 2:** Mean value glandular trichome on the adaxial and abaxial side of leaves for selected species of family Lamiaceae Martinov

High degree of variability has been found with peltate glandular trichomes size of *Thymus serpyllum* species, where the variability degree exceeded 20%. A moderate variability degree (from 10 to 20%) of glands size showed the species *Hyssopus angustifolius*, *Hyssopus officinalis*, *Satureja montana*, *Ocimum basilicum* and *Nepeta transcaucasica*. Low degree of variability (up to 10%) of glands size has been detected by species *Thymus vulgaris*, *Salvia officinalis*, and *Monarda fistulosa*.

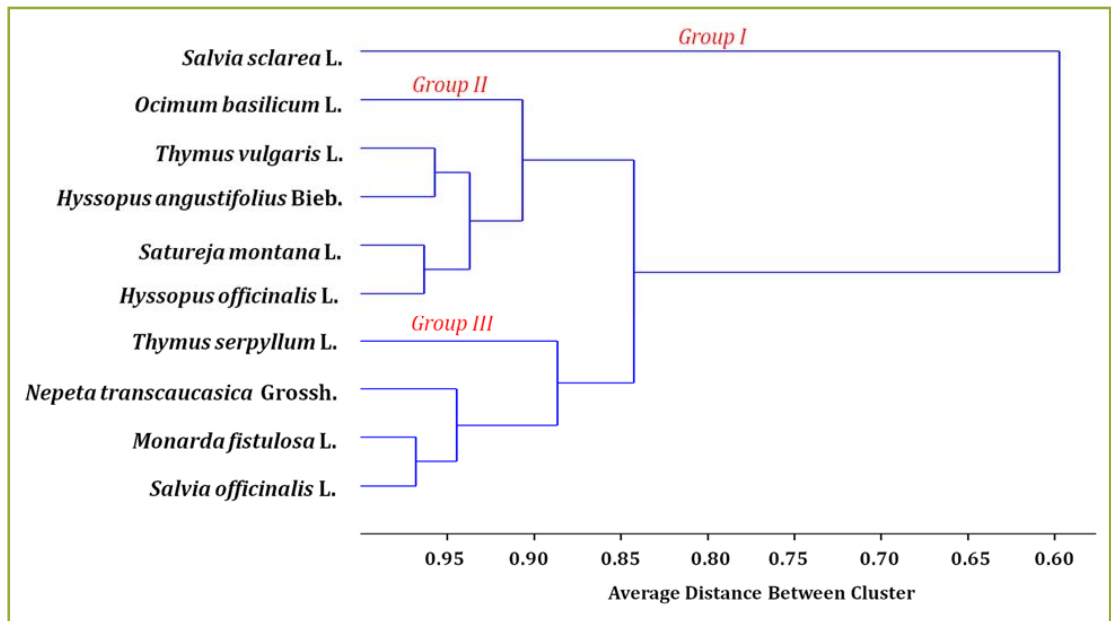
The adaxial surface of *Thymus vulgaris* leaves has more glandular trichomes of lamiaceous type compared to the abaxial surface of ones. The two surfaces *Thymus serpyllum* leaves contain approximately the same quantity of these glandular trichomes. The numerous glandular trichomes of the lamiaceous type of amber or reddish brown colour had a shiny spherical shape. However, it was noted that *Thymus serpyllum* had significantly less of these glandular trichomes with yellow or brown colour compared to *Thymus vulgaris* that could be explained by less yield of essential oil (minimum 12 mL.kg<sup>-1</sup>) for *Thymus vulgaris* (anhydrous drug) and minimum 3.0 mL.kg<sup>-1</sup> of essential oil for whole or cut, dried, flowering aerial parts of *Thymus serpyllum* (European pharmacopoeia, 2016).

Our study also demonstrated that two surfaces of *Salvia officinalis* leaves had approximately the same quantity of these glandular trichomes. However, it was noted that the leaf adaxial side of *Salvia sclarea* had no glandular trichomes of lamiaceous type at all. Such a difference could be explained by less yield of essential oil (0.19–0.38%) for *Salvia sclarea* and minimum 15 mL.kg<sup>-1</sup> of essential oil for the whole dried drug or minimum 10 mL.kg<sup>-1</sup> of essential oil for the cut drug (anhydrous drug) of *Salvia officinalis* (European pharmacopoeia, 2016).

The feature of the glandular trichomes of lamiaceae type of *Monarda fistulosa* L. is their location. They are located below of epidermis surface in the mesophyll that conforms with studies conducted by Shanaida et al. (2016).

The feature of the glandular trichomes of lamiaceae type of *Hyssopus angustifolius* Bieb. is a distinction in their sizes on both leaf sides. These glandular trichomes are significantly larger on the adaxial side. While glandular trichomes of lamiaceae type of *Hyssopus officinalis* are the same size on both leaf sides.

Based on cluster analyze was done a dendrogram (Figure 3). In this study, 10 species were grouped into three main clusters based on the highest similarities.



**Figure 3** Cluster dendrogram based on morphometrics parameters adaxial and abaxial surfaces of leaves for selected species of family Lamiaceae Martinov

On the dendrogram, it can be seen that *Salvia sclarea* (Group I) really differs from the other samples because peltate glandular trichomes on the adaxial leaf surface have not been determined. Only simple trichomes were found. *Salvia sclarea* formed peltate glandular trichomes only on the abaxial leaf surface. Group II included the species which had the least signs comparing with Group III.

## Conclusion

The tested plant species showed significant differences in size and colour of peltate glandular trichomes producing essential oils. This difference can be used during macroscopic analysis of herbal substances. Aromatic plants that were grown in Steppe zone of South Ukraine are perspective plants because of presence a well-generated excretory system which contains essential oils. It is planned to continue the investigation of morphological and anatomical properties of peltate glandular trichomes with essential oils and to conduct a study of chemical analysis of the essential oils.

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