THE HISTOCHEMICAL ANALYSIS OF THYMUS MALYI RONNINGER GLANDULAR TRICHOMES

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ABSTRACT

The genus Thymus L. belongs to the family Lamiaceae, and comprises numerous aromatic species which are used for medical purposes and also as culinary herbs. Thymus malyi Ronninger is an endemic species from central Balkan which grows on serpentine hills. Histochemical analyses of the peltate and capitate glandular trichomes of Thymus malyi were carried out using light microscopy. Results of histochemical tests showed positive reaction to phenol compounds in the secretory heads of peltate trichomes. Positive reaction for terpenes was obtained in capitate trichomes and in the subcuticular spaces of peltate trichomes. Lipid reaction was positive in the stalk cell of the capitate trichomes, and in the peltate trichomes heads. Positive reaction for polysaccharides was observed in the secretory head of both types of glandular trichomes, with more intensive reaction in the capitate trichomes.

Keywords: Thymus malyi, glandular trichomes, histochemistry

Introduction

Trichomes are specialized epidermal cells found on the surfaces of nearly all plants. They possess a variety of characteristics and serve different functions, such as reflecting radiation, lowering plant temperature, reducing water loss (11), or providing defense against insects. Trichomes found in different species can vary in their structure, being unicellular or multicellular, branched or unbranched. Trichomes with secretory function are glandular, consisting of a stalk terminating with a glandular head. The Lamiaceae is a family rich in aromatic species used as herbs, folk medicines, fragrants, etc., because of the essential oils produced in glandular hairs spread over the aerial vegetative and reproductive organs. In the Lamiaceae, two types of glandular trichomes, peltate and capitate, were identified (12).

The genus Thymus L. (Lamiaceae) comprises numerous species and varieties, with medicinal and culinary usage, therefore their essential oil compositions have been studied intensively (1, 3, 9, 10).

Thymus malyi Ronninger is an endemic species from central Balkan which grows on serpentine hills. It is low creeping shrub, with ovate leaves and capitate inflorescence with purple corolla (5).

The purpose of the presented study was to investigate the histochemistry of the secretion products of glandular trichomes on the leaves of T. malyi using a light microscope.

Materials and Methods

Aerial parts of T. malyi were collected at the flowering stage in June 2006, in the southwest Serbia (Studenica) and west Serbia (Ibar-Kula). Voucher specimens were deposited in the Herbarium of Institute of Botany, Faculty of Biology, University of Belgrade. Histochemical analyses were performed on hand-sections of fresh leaves using the following tests: FeCl3 for phenols (6), NADI for terpenes (4), Sudan IV for lipids (8), PAS for polysaccharides (7). Standard control procedures were carried out simultaneously. The observations were made under a Leitz Dialux light fluorescence microscope HBO 50W block filter A-excitation
wavelengths were BP 340-380.

**Results and Discussion**

The leaves of *T. malyi* are covered with a thick cuticle on the adaxial leaf side. Epidermis, as unicellular layer, is present at the abaxial and adaxial surface. Two types of glandular trichomes - peltate and capitate were found. The peltate trichomes, located in epidermal depressions, were composed of one basal cell, short stalk cell and a rounded head. The capitate glandular trichomes are composed of one basal cell, a short stalk cell and unicellular head.

The results of histochemical analysis of the secreted products of the glandular trichomes are presented in Table 1.

**TABLE 1**

<table>
<thead>
<tr>
<th>Staining procedure</th>
<th>Target compounds</th>
<th>Peltate trichomes</th>
<th>Capitate trichomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeCl₃</td>
<td>phenols</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>NADI</td>
<td>terpenes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sudan IV</td>
<td>lipids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PAS</td>
<td>polysaccharides</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Legend: (+) - positive reaction; (-) - negative reaction

For the histochemical analysis of the secreted material, several staining methods were used. Results of histochemical tests showed positive reaction to phenolic compounds (Fig. 1) in secretory heads of peltate glandular trichomes, with dark brown colour. Staining with NADI reagent for terpenes (Fig. 2) was positive, showing blue color in the secretory head of capitate trichome. Sudan IV (Fig. 3) for lipids gave a positive reaction showing dark red colour in the stalk cells of capitate trichomes, while red staining with PAS (Fig. 4) gave positive reaction for polysaccharides and was observed in both types of glandular trichomes, showing more intensive reaction in the capitate trichomes heads.

![Fig. 1. Dark brown staining of secretion with FeCl3 in peltate trichomes 100x](image1)

![Fig. 2. Blue staining of secretion with NADI in capitate trichomes 100x](image2)

![Fig. 3. Dark red staining of secretion with Sudan IV in capitate trichomes 40x](image3)
The examined stains showed certain differences in colour between peltate and capitate trichomes, but we can not conclude about exact differences without detailed chemical analyses.

The representatives of the Lamiaceae family are characterized by presence of peltate and capitate glandular trichomes. The secreted material of heterogeneous composition is temporarily stored in the subcuticular space of the mature peltate trichomes and released by rupture of cuticle, while in the capitate trichomes it is probably released through micropores (12). Their secretions may be involved in the chemical defense of plants, or in pollinators attraction, but the specific composition of secreted material in many Lamiaceae species have been investigated because of the biological effects of essential oils, which are widely used in pharmaceutical preparations, perfumery and cosmetics (2, 12). Taxonomically, Thymus is a very complex genus, because of the polymorphism of a number of species and the absence of intrageneric incompatibility. Interspecific and unprogressive hybridization between related species is a very common feature of this genus and the main cause of variation. Data from histochemical tests revealed that the secreted material in the glandular trichomes of Thymus malyi is of heterogeneous composition, containing polysaccharides, unsaturated lipids and proteins.

Our investigation, comparing to the previously published results, has shown a lot of similarities in histochemical features of secreted contents of glandular trichomes with other investigated species belonging to the family Lamiaceae. Considering the Thymus species, which are taxonomically complex group of Lamiaceae, further research which will include more detailed histochemical, ultrastructural and chemical analyses in order to find some differences among the species, is needed.

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REFERENCES