GLANDULAR TRICHOMES AND ESSENTIAL OIL COMPOSITION OF *Thymus pannonicus* All. (*Lamiaceae*)

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Abstract. Glandular trichomes of *Lamiaceae* are among the most investigated secretory structures. Micromorphological and anatomical analyses of the glandular trichomes of *Th. pannonicus* L. were carried out using SEM (Scanning Electron Microscopy). Our research results show that, the secretory structures are always multicellular, consisting in a basal cell, a unicellular pedicel and a gland which bears 1, 2, 8 or 12 cells. Aerial parts of *Th. pannonicus* L. were subjected to hydrodistillation to yield volatile oil which were subsequently analysed by GC/MS (gas chromatography coupled with mass spectrometry). The main compounds identified in *Th. pannonicus* L. volatil oil were α-terpinyl acetate (48.83%), germacrene D (12.12%), cariophyllene oxide (6.35%) and mircene (4.73%).

Keywords: *Thymus pannonicus* L., glandular trichomes, micromorphology, volatile oil.

INTRODUCTION

The *Lamiaceae* is a large plant family rich in aromatic species used as culinary herbs, in folk medicines, in pharmaceutical industries etc. Many species of this family possess volatile oils secreted by the glandular trichomes. The studies in which the glandular thichomes have been investigated, comprising morphological, structural and histochemical analysis of trichomes as well as chemical composition of volatile oils [1, 2, 4, 6, 13, 17, 18, 19].

Among the aromatic plants belonging to the family *Lamiaceae*, the genus *Thymus* is noteworthy for the numerous species and varieties of wild-growing plants. Many of these species are typical for the Mediterranean area [5]. It is estimated that the genus *Thymus* comprises at least 350 species, with many subspecies, varieties and form, endemic and widespread, distributed over Europe, Asia, North Africa and the Canary Islands [12]. In Romania the genus *Thymus* is represented by 16 species and 12 hybrids [14]. *Thymus* species are used as medicinal and aromatic plants, as well as in cosmetics and perfumery. Most aspects of their medicinal use are related to the volatile oils [10].

Thymus pannonicus All. is a perennial herbaceous plant, distributed in central and eastern Europe as well as in Russia, over open dry grasslands and rocks. In Romania this species presents vigorous branched stems, covered with hairs with the same length of the axis diameter. The leaves are elliptic or prolonged, 6-12 mm in long and 3-5 mm wide, green in color, both epidermis are covered with hairs, nervures little proeminent. The inflorescence is capitate. The calyx is 3-4 mm long, the corolla is lilac-red, 6-7 mm long [7].

The dried herb of *Th. pannonicus* All. is used to make tasty and refreshing herbal tea drinks, due to its peculiar and pleasant lemon-likes scent. Fresh leaves are used for aromatization of homemade jams, candies and similar confections. It is also been taken with positive results for coughs and other respiratory complaints, as well as some cases of gastrointestinal disorders.

The aims of this investigation is: (I) to analyze the micromorphological peculiarities of the glandular hairs of this species and (II) to determine the chemical composition of the volatile oil.

MATERIALS AND METHODS

The aerial parts of *Thymus pannonicus* All. were collected during the flowering period from Suceava district.

Micromorphological observations were carried out on stems and leaves using Scanning Electron Microscopy (SEM) in the Department of Electronic Microscopy, University of Vigo, Spain. The 1 cm^2 samples were fixed using glutaraldehyde and osmium tetraoxyde and dehydrated using ethylic alcohol (35%, 50%, 70%, 90%, 95%, 15 minutes each and absolute alcohol 3 times for 20 minutes each time), followed by anhydric acetone. The coatings of the dehydrated samples has been carried out using gold in a 30-60A thick layer and were observed with a Philips XL30 SEM microscopy.

The volatile oil has been extracted using a Clevenger type apparatus according to the European Pharmacopoeia standards, in the Laboratory of Plant Physiology, Faculty of Biology, Iasi.

The separation and the identification of the components have been carried out with GC-MS (gas chromatography coupled with mass spectrometry) at the University of Agronomic Sciences and Veterinary Medicine, Bucharest.

RESULTS

Micromorphological peculiarities.

The leaves of *Th. pannonicus* All. are covered with a thick cuticle. A uni-layered epidermis is present on the abaxial and adaxial surface. In a front side view the epidermis consists in irregular-shaped cells, with waved lateral walls. The stomata of diacytic type are present in both epidermises, so the limb is of amfistomatic type. The leaves have non-glandular and glandular trichomes on both sides of the lamina (Fig. 1 a, b). Non-glandular trichomes are present on the veins and leaf margins and they are unbranched.

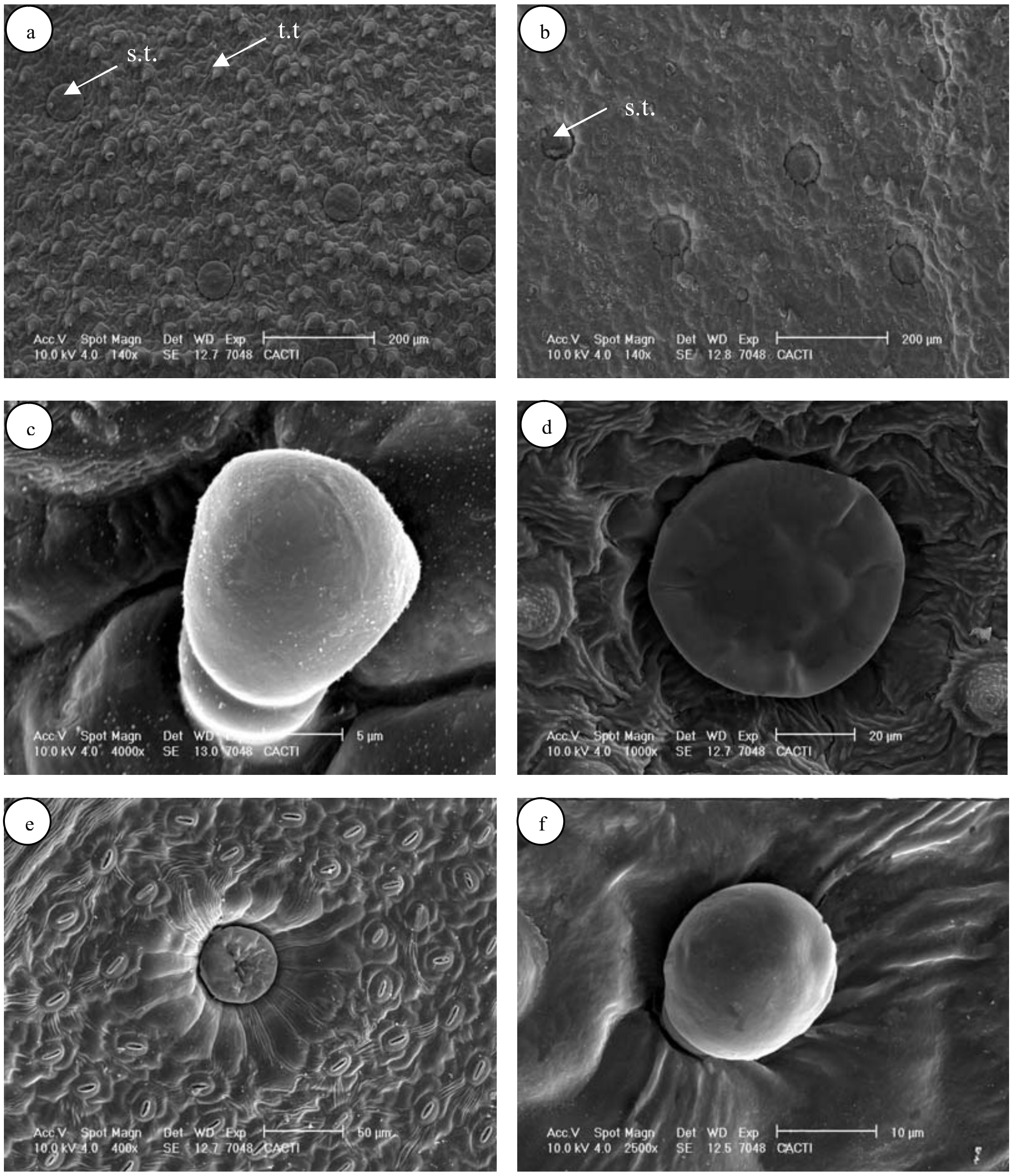

Figure 1 Secretory trichomes in *Th. pannonicus* All.: a. adaxial side of leaf: s.t. – secretory trichome, t.t. – tectorial trichome; b. abaxial side of leaf: s.t. – secretory trichome; c. secretory hair with a unicellular gland on the adaxial side of leaf (type A); d. secretory hair with a pluricellular gland on the adaxial side of leaf (type b); e. secretory hair with a unicellular gland on the abaxial side of leaf; f. secretory hair with a pluricellular gland on the abaxial side of leaf.

The glandular trichomes observed in the examined plant can be grouped as follows: secretory hairs with a unicellular gland - type A and secretory hairs with a pluriccellular gland – type B.

Types A consist of a basal epidermal cell, a short neck cell and the gland is formed by a single cell (Fig. 1c, g). Type B consists of a basal epidermal cell, a short neck cell and the gland is form by 2 or more cells (Fig. 1d, f). The glandular trichomes with unicellular gland are more numerous than the ones with pluricellular gland. In front side view, the epidermic cells of the limb, which surround the secretory trichomes, are radialy elongated towards the cuticle and bear uncurved walls (Fig. 1f).

On the stem, the secretory trichomes are short, formed by of a uni- or pluricellular gland. The secretory trichomes with pluricellular gland are not located in deep concavities, like the ones from foliar blade and their frequency rises from the basis to the apex of the organ (Fig. 2 a, b, c).

The secreted material is stored in a large subcuticular space, originated by the detachment of the external part of the wall on the glandular head, and released along with the cuticle rupture (Fig. 2d).

Chemical composition of the essential oil.

GC-MS analyses of *Th. pannonicus* essential oil resulted in the identification of 25 compounds.

The main constituents are: α-terpinyl acetate (48.83%), germacrene D (12.12%), cariophyllene oxide (6.35%) and mircene (4.73%). All components are listed in Table 1, in order of their elution.

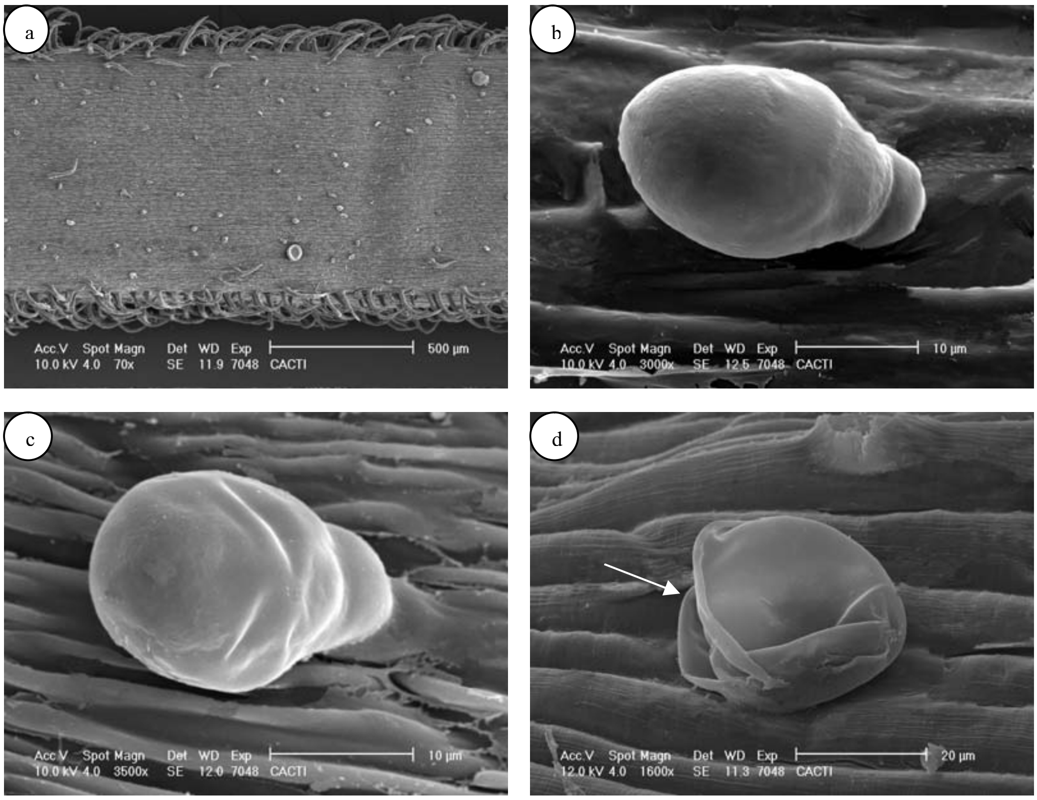

Figure 2. Secretory trichomes in *Th. pannonicus* All. a. stem; b. secretory hair with a unicelular gland from the superior part of the stem; c. secretory hair with a unicelular gland from the middle part of the stem; d. secretory hair with a pluricelular gland of stem.

Table 1. Chemical composition of *Thymus pannonicus* volatile oil.

No.	Compounds	%	No.	Compounds	%
1.	Sabinene	0.34	14.	Neryle acetate	2.21
2.	**Mircene**	**4.73**	15.	α - Burbonene	0.98
3.	Octinyl acetate	1.16	16.	ß-Cariophyllene	1.32
4.	Limonene	2.69	17.	**GermacreneD**	**12.12**
5.	cis-ß-Ocimene	2.61	18.	Elemene	0.58
6.	p-Cymene	0.59	19.	ß-Bisabolene	1.14
7.	α - terpineole	0.44	20.	δ-Cadinene	0.69
8.	Cis-Geraniol	0.30	21.	Spatulenol	2.29
9.	ß - Citral	0.89	22.	**Cariophyllene oxide**	**6.35**
10.	α - Citral	1.20	23.	α – Cadinol	1.09
11.	Trans-Geraniol	1.46	24.	Izoaromodendren epoxid	0.53
12.	**α -Terpinyl acetate**	**48.83**	25.	Trans-Farnesol	1.45
13.	Geranyl acetate	0.5	-	-	-

The main constituents of the essential oil are responsible for medicinal and non-medicinal activities. α -Terpinyl acetate represent 48,83% and according to the literature present antibacterial activity [11]. Germacrene D is a hydrocarbon from sesquiterpene class and it can be used as a pesticide and pheromone [20]. The cariophyllene oxide has anti-inflammatory proprieties [15] and also it can be used as insecticide and fungicide [3]. The mircene is a monoterpene utilized in perfumery and also in medical purposes as an analgesic, fungicide and antibacterial substance [8].

DISCUSSIONS

In conclusion the Scanning Electron Microscopy reveled the fact that: (I) in *Th. pannonicus* we have two trichome types: tectorial and glandular, and the first are multicellular; (II) the glandular trichomes are always multicellular, having instead an unicellular base, an unicellular stalk and the gland composed of 1, 2 or 8 cells.

Concerning the chemical composition of the volatile oil, in a survey of available literature, only few publications relevant for the chemical composition of *Th. pannonicus* were found, suggesting that a substantial lake of information in this field still exists. A greater interest in this essential oil was expected as this species is neither endemic, nor endangered across the whole area of its distribution. According to Karuza-Stojaković *et al.*, [8] the principal constituents of *Th. pannonicus* essential oil from southern parts of Vojvodina province were terpinyl acetate, terpinen-4-ol, thymol, carvacrol and geranyl acetate (listed in order of descending quantity). Recent comprehensive studies of chemical variability in hydrodistilled volatil oils of different wild growing and cultivated populations of *Th. pannonicus* from Hungary, aswell as supercritical fluid extracts of various *Lamiaceae* species, confirmed that high concentrations of both thymol and *p*-cymene are the main chemosystematic attributes of *Th. pannonicus* essential oil [14].

The chemical composition of volatile oils of *Thymus* species present a high variability and diversity, at least 20 different chemotypes have been established thus far [16]. The production of phenolic compounds is higher in warmer and drier climatic zones; non-phenolic compounds usually accumulate in higher quantities in colder and damper areas [9].

Z. Maksimović et al. (2008) found in the volatile oil of *Th. pannonicus* 33 compounds, the main constituents being geranial (41.42 %) and neral (29.61); these compounds were not found in the volatile oil of the plant that we have analysed.

Other authors found in the volatile oil of this species big quantities of tymol (25-41 %) and p-cimene (17-38%) [14]. We identified only p-cimene, but in small quantities (0.59%).

Th. pannonicus tolerate a variety of substrates and vegetation types but prefers a slightly acidic or neutral pH and eleveted levels of Ca, Mg, Mn, Fe, Co or Cr [12], this can be a explain for the chemical variability of this species.

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