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## Anatomical observations on *Thymus bracteosus* Vis. ex Bentham (*Lamiaceae*)

### Abstract

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*Thymus bracteosus* Vis. ex Bentham, endemic to the Mediterranean and submediterranean mountains of Croatia, Herzegovina and Montenegro, has been analyzed as regards the anatomy of stem, leaves and bracts. Its structure shows xero-mesomorphic characteristics. The quantity of essential oil extractable for commercial purposes is comparatively low.

### Introduction

*Thymus bracteosus* Vis. ex Bentham belongs to the sect. *Hyphodromi* (A. Kerner) Halácsy section (Jalas 1972).

It is endemic to the Dinaric area, with a very limited distribution in the central Balkan peninsula (Croatia, Herzegovina, Montenegro) (Fig. 1).

*T. bracteosus* is a perennial herb with long, procumbent stems forming more or less large clusters. The flowering stems are up to 10 cm high, woody, pubescent. The leaves are oblong-spathulate, obtuse on the top, entire on the margins, glabrous, ciliate in the basal part, 10-17 (20) mm long and 2-3 mm wide. The bracts, a significant characteristic of this species, are large, up to 13 mm long and 6-7 mm wide, ovate, long-ciliate at the margins. The inflorescence is capituliform about 1.5 cm in diameter. The calyx is (4) 5-6 mm long, with the sepals sharply toothed on the top. The corolla is 6-8 mm, purple, strongly 2-lipped, the upper lip entire. There are 4 stamens, their upper parts widely separated. It blooms from July to August.

*T. bracteosus* inhabits different communities of the *Scorzonero-Chrysopogonetalia* Horvatic et Horvat order. It is spread in bare dolomite vegetation, on open meadows and carbonate rocky grounds of the mediterranean and submediterranean region (Silic 1984).

The anatomic structure of *T. bracteosus* had not yet been studied. The aim of this work is therefore to describe the anatomy of the stem, leaves and trichomes of this species and to point out its important and adaptive characteristics.

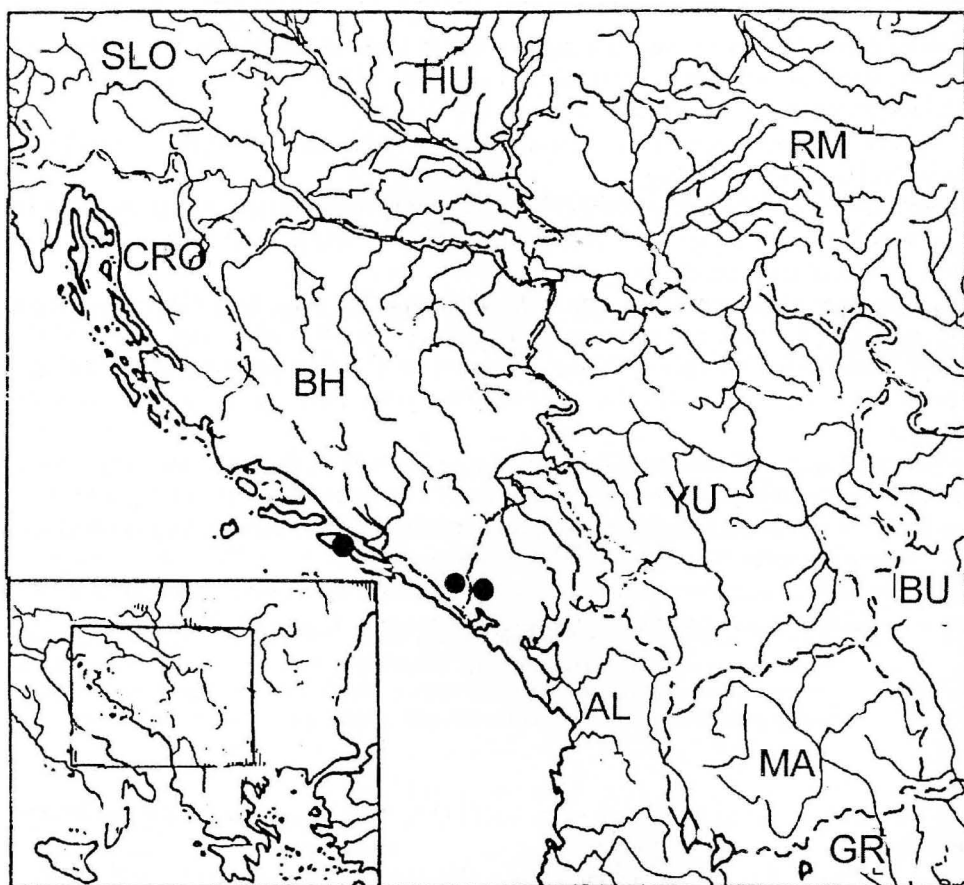


Fig. 1. Distribution of *Thymus bracteosus* Vis. ex Benth.

Considering that the species of the genus *Thymus* are well-known as medicinal and aromatic plants because of their essential oil (Morales 1996), the aim is also to identify the drug and to investigate the quantity of essential oil in the above-ground shoot of *T. bracteosus*.

#### Material and methods

The plant material was gathered on the Prčanj locality, on the mountain Orjen (Montenegro), 900 m, N. E. exposition, inclination 15°. In this habitat *T. bracteosus* occurs on the sunny, dolomite and limestone-dolomite rocky grounds and dry meadows where it is a member of the *Lino-Salvietum brachyodoni* Silic & Abadzic, endemic association, which belongs to the vegetation of the *Saturejon subspicatae* Horvat suballiance (Abadzic & Silic 1982).

The soil type alternates from dolomite serozem to rendzina.

The local weather conditions are those of perhumid oro-mediterranean climate (Horvat & al. 1974). Average yearly temperature is 8.7°C. High temperatures and low relative air

humidity can appear in the summer months which leads to the occurrence of drought. Absolute minimal temperatures appear all year round except during the summer, from June to September (Pavlovic & al. 1984).

Voucher specimens have been deposited at the Department of Botany, Faculty of Pharmacy, University of Belgrade.

Permanent microscopic preparations were made of plant material fixed in the field in 60% alcohol. Cross sections were obtained by cutting on sliding microtome, and double stained with safranin and alcian blue.

The diameter of the stem (cortex, central cylinder, pith), the height of the leaf and bract epidermal cells, the thickness of the palisade tissue, the number of the layers of palisade and the thickness of the spongy tissue were measured on the permanent preparations, under a light microscope. All the measurements were made in a software package "OZARIA".

The morphology of epidermal hairs on the leaves was analyzed in two ways: by a binocular magnifying-glass on herbarium material - paradermal section preparations and by a light microscope on cross sections made of fixed plant material. Epidermis peels were made using Jeffrey's solution.

The essential oil was extracted from dry above-ground parts of the flowering plant by vapour distillation in an apparatus designed by Clevenger (Ph. Jug. IV 1984).

## Results

### *Stem*

In cross section the stem is rectangular with lightly bulging angles and convex lateral sides (Fig. 2).

The epidermis has one layer of large cells with a rugged cuticle and simple, unbranched, unicellular and multicellular hairs. Below the epidermis there is collenchyma tissue, well developed in the angles. Cortex parenchyma is thin-walled, with small intercellular spaces. The inner layer of cortex parenchyma is represented with one continuous ring of cells with thin walls which form the endodermal layer.

The phloem, cambium and xylem develop into cylinders, and the xylem is three times wider than the phloem. Pith parenchyma is strongly developed, the diameter of the pith varies from 201-255.6  $\mu\text{m}$ , filling the stem to the central cavity and is formed by large cells with thin walls with numerous intercellular spaces.

### *Leaf*

The leaves are dorsiventral. Sparse indumentum appears on the surface, made of two types of trichomes:

- non-glandular trichomes sporadic both on the upper and lower side
- glandular capitate and peltate hairs (Fig. 3a).

The cuticle is well developed and forms many creases on the epidermis surface.

The epidermal cells (Fig. 3) of the upper side are larger (27.1-40.1  $\mu\text{m}$  in height) than those of the lower side (14.8-32.5  $\mu\text{m}$  in height).

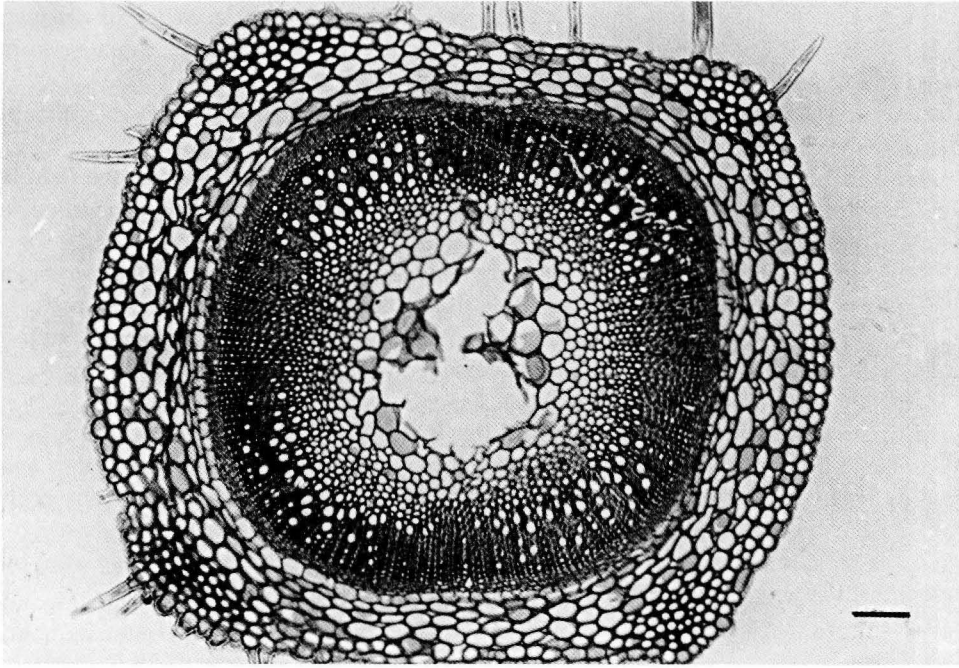


Fig. 2. Cross section of the stem — Scale bar = 100  $\mu$ m.

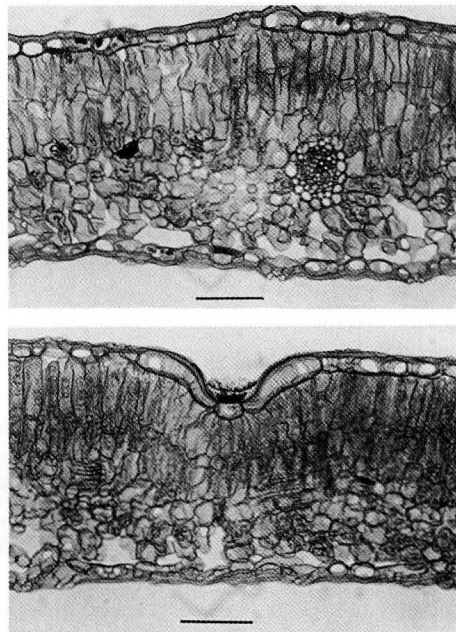


Fig. 3. Cross section of the leaf; **3a**. peltate hair.— Scale bar = 100  $\mu$ m.

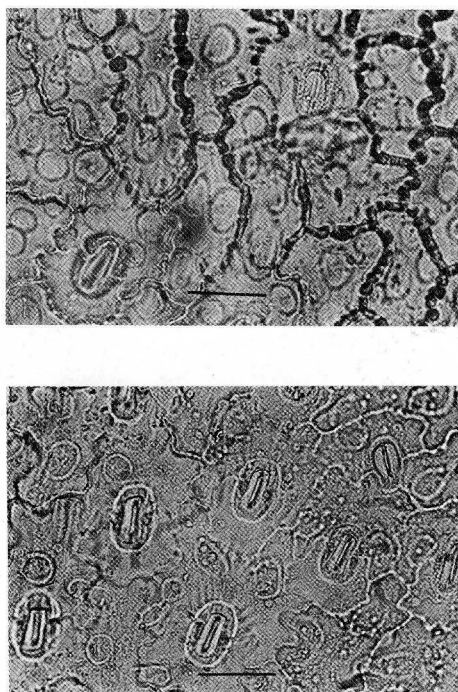


Fig. 4. Paradermal sections of the leaf epidermis: **a**, upper side; **b**, lower side. — Scale bar = 50  $\mu\text{m}$ .

The anticlinal cell walls are sinuous, more distinctly so on the upper (Fig. 4a) than on the lower side of the leaf (Fig. 4b). The outer tangential walls are thickened.

The leaves are amphistomatic, with diacytic stomata (Fig. 4a, b) slightly raised above the epidermis level (Fig. 3, 3a) and more numerous on the lower side of the leaf.

The mesophyll is differentiated into palisade and spongy parenchyma (Fig. 3). The palisade parenchyma consists of two to three layers, 134.3-197.2  $\mu\text{m}$  thick. The radial walls of the palisade cells are slightly sinuous. The spongy parenchyma consists of 3-4 layers (111.4-163.8  $\mu\text{m}$  thick) of cells of irregular form, relatively compact, with small intercellular spaces. Palisade and spongy tissue ratio is 1 : 1.

#### *Bract*

There are non-glandular and glandular hairs on the epidermis. The non-glandular hairs are unicellular and multicellular, unbranched. The non-glandular bracteal hairs are distributed more densely than those on the leaf (Fig. 5). Capitate and peltate hairs can be found both on the upper and lower side, but are more numerous on the lower side.

The cuticle is striated and well developed (Fig. 6a).

The epidermis has one layer. The cells of the upper side are somewhat larger (15.6-28.6  $\mu\text{m}$  thick), with a very thickened outer tangential cell wall. The anticlinal cell walls of the upper side are more sinuous (Fig. 6a) than those of the cells of the lower side (Fig. 6b). The epidermis of the lower side is composed of cells 10.4-33.8  $\mu\text{m}$  in thickness.

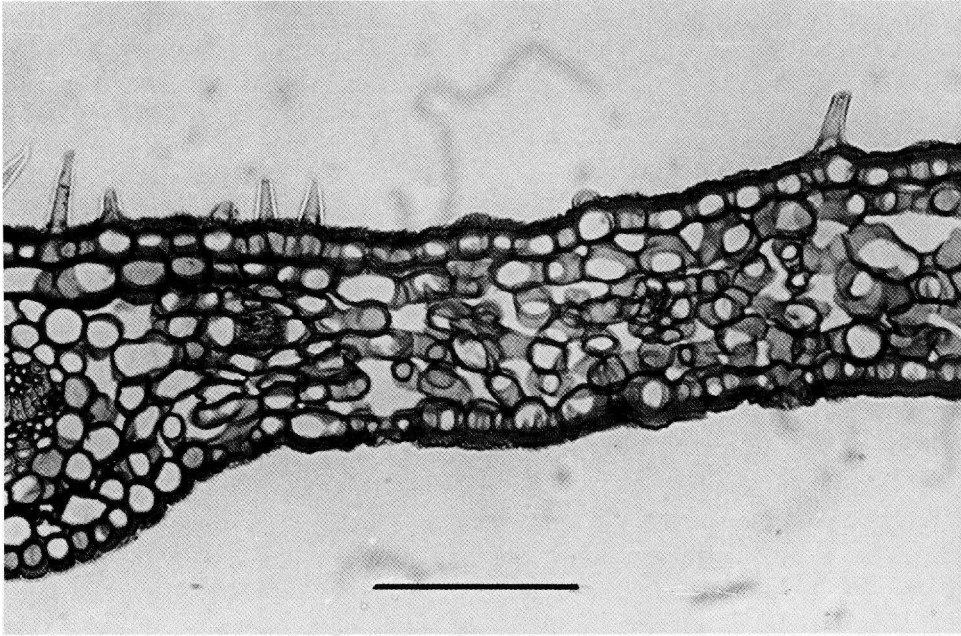


Fig. 5. Cross section of the bract. — Scale bar = 100  $\mu$ m.

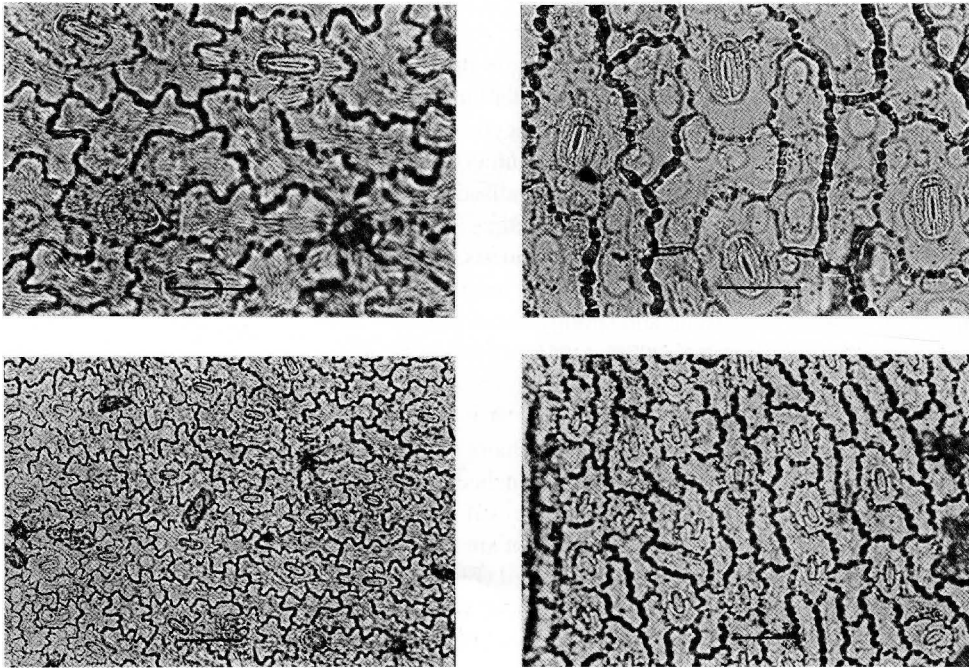


Fig. 6. Paradermal section of the bract epidermis: **a**, upper side; **b**, lower side. — Scale bar = 50  $\mu$ m.; **c**, upper side; **d**, lower side. — Scale bar = 100  $\mu$ m.

The stomata are diacytic, in the epidermis level, 540.6-952.1  $\mu\text{m}$  in surface on the upper side and 311.2-517.1  $\mu\text{m}$  on the lower (Fig. 6c, d). They are more numerous on the lower side.

The mesophyll ranges from 74.7 to 127.3  $\mu\text{m}$  in thickness. It is not differentiated into palisade and spongy tissue, is formed by cells of irregular shape and includes numerous intercellular spaces (Fig. 5). The vascular bundles are collateral with parenchyma toward the upper side of bract and fibres toward the lower side. The bundle is wrapped with a vascular parenchyma sheath.

#### *Essential oil*

The essential oil extracted from the dry above-ground shoot of the flowering plant is 0.15%.

#### **Conclusions**

According to the histological structures of its vegetative organs, the endemic species *Thymus bracteosus* is similar to other representatives of the genus *Thymus* (Metcalf & Chalk 1950, Marhuenda & Alarcon de la Lastra 1987, Zivanovic 1977, 1979).

The anatomical structure of the vegetative organs of *T. bracteosus* displays xeromorphomorphic characteristics which can be considered as an adaptation to open limestone habitats of the mediterranean and submediterranean regions where this species occurs. Distinct xeromorphism can be seen in:

- thick cuticle on all the above-ground parts of the plant (stem, leaves, bracts)
- thick outer tangential walls and slightly sinuous anticlinal epidermal cell walls
- glandular and non-glandular hairs increasing the external protection of cuticle
- relatively thick lamina to 360  $\mu\text{m}$
- sinuous radial cell walls of the palisade tissue.

Characteristics of the mesomorph structure are:

- leaf mesophyll differentiated into palisade and spongy tissue in ratio of 1 : 1
- stomata raised above epidermis level

Bracts show a different structure compared to the leaf:

- non-glandular and glandular hairs are more numerous
- mesophyll is not differentiated into palisade and spongy parenchyma.

The essential oil content is relatively small (0.15%). Other species of the *Hyphodromi* section contain a larger amount of essential oil: *T. cilicicus* Boiss. Ball. 1.5%, *T. sipyleus* Boiss. 1.2%, *T. zygioides* Griseb. 0.62% (Ilisilu & Tanker 1986), *T. leptophyllus* Lange 0.8% (Blázquez & al. 1989), *T. atticus* Celak 0.64% (Tümen & al. 1997). In *T. bracteosus* the presence of peltate and capitate hairs, especially on the bracts, points out that these secretory trichomes could represent the place of the synthesis of essential oils but also of other secondary metabolites.

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