

Glandular trichomes on the leaves of *Rosmarinus officinalis*: Morphology, stereology and histochemistry

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Abstract

Stereological and histochemical analyses of the glandular trichomes on leaves of *Rosmarinus officinalis* were carried out using light and fluorescence microscopy. Non-glandular and two types of glandular trichomes – peltate and capitate – are described. The stereological method was used for estimating the volume density of epidermis, mesophyll, mechanical tissue, central cylinder, intercellular spaces and volume density of different types of glandular trichomes. The results showed that the volume density of adaxial epidermis was higher than abaxial epidermis. The volume density of peltate trichomes was higher than the volume density of capitate ones. The values obtained for number of peltate and capitate trichomes showed that the capitate trichomes type I were more numerous. The histochemical tests showed positive reactions to proteins and polysaccharides for both types of trichomes, while the phenolic substances were found only in peltate trichomes.

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1. Introduction

Plants produce a large number of secondary compounds that are utilized as pharmaceuticals, nutraceuticals, natural pesticides, flavourings, fragrances and for other non-food or fiber purposes. These compounds have diverse biological activities, presumably as a result of the co-evolution of the producing plants with pathogens, herbivores, pollinators and other organisms (Duke, 1994).

The Lamiaceae is a large family, rich in aromatic species used as culinary herbs, folk medicines, fragrances, etc. Many species of this family possess essential oils secreted by glandular trichomes. In the family Lamiaceae, there are two main types of glandular trichomes: peltate and capitate. The studies in which these glandular trichomes have been investigated, comprising morphological, structural and histochemical analyses of trichomes as well as chemical composition of essential oils are still rare (Amelunxen, 1964; Werker and Fahn, 1981; Bosabalidis and Tsekos, 1982; Werker et al.,

1985a,b; Dudai et al., 1988; Antunes and Sevinate-Pinto, 1991; Ascensão et al., 1995).

Rosmarinus officinalis L. is an evergreen, perennial shrub, native to the chalky, calcareous hills along the Mediterranean Sea. Dried rosemary leaves are used as seasonings for soups, meat, fish and poultry. The oil extracted from flowering tops, stems and leaves is used in food products, perfumes, and cosmetics, such as soaps, creams, deodorants, hair tonics, and shampoos. The plant and extracts possess antibacterial and antioxidant activity and it is considered good source of nectar for bees. As a medicinal plant, rosemary has been used as an external stimulant and as a relaxant for nervousness, muscle spasms, and headaches. It has also been used in the treatment of cancer, and is categorized today as a therapeutic emmenagogue (Simon et al., 1984).

Stereological methods with useful parameters such as volumes, surface area, number of particles (Toth, 1982) have in the last few decades been applied in research of plant species (Kubinova, 1989, 1991, 1993). Since there are no available data about stereological investigation of glandular trichomes in the Lamiaceae family, the stereological method was used for the estimation of volume density and number of morphologically distinct types of glandular trichomes of *R. officinalis*. The aim

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of this study was also to investigate the morphology, distribution, and histochemistry of glandular trichomes *R. officinalis* and to compare our results with the previous observation reported by Werker et al. (1985a).

2. Material and methods

The plant material for this study was collected in the nursery garden in area of Pančevo City in spring 2003. Five leaves from five different plants and three sections per leaf at 5 mm distances were used for stereological examination. Three sections per leaf were taken although Kubinova (1993) suggested five sections, because leaves of *R. officinalis* are linear and morphologically rather than uniform from the base to the apex. Leaves were sectioned in 2% glutaraldehyde and put into a fixative solution of 2% glutaraldehyde in phosphate buffer (0.1 M, pH=7.0), post fixed in 2% OsO₄ and embedded in Araldite. Sections of 1 μm thickness were stained in toluidine blue. Weibel's multipurpose test grid (M 42) was used to estimate volume density and number of morphologically distinct types of glandular hairs of *R. officinalis* (Weibel et al., 1966). Stereological measurements were done using an Olympus CX-41 light microscope.

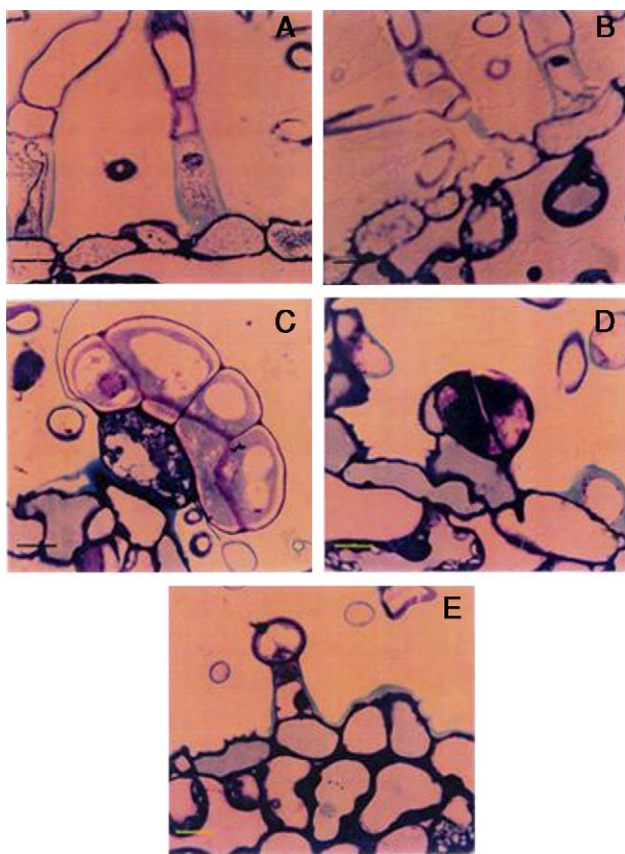


Fig. 1. Light micrographs of glandular trichomes of leaves of *Rosmarinus officinalis*: (A) non-glandular unbranched trichomes in longitudinal section, bar=100 μm; (B) non-glandular branched trichomes in longitudinal section, bar=100 μm; (C) peltate glandular trichomes in longitudinal section, bar=100 μm; (D) capitate glandular trichomes type I in longitudinal section, bar=100 μm; (E) capitate glandular trichomes type II in longitudinal section, bar=100 μm.

Table 1

Stereological results obtained of the leaf volume density (mm⁰) of *Rosmarinus officinalis* (mean±S.E.M.)

	Volume density (mm ⁰)
Epidermis adaxial	0.04±0.003
Epidermis abaxial	0.03±0.002
Central cylinder	0.04±0.001
Mesophyll	0.30±0.007
Intercellular spaces	0.24±0.008
Mechanical tissue	0.32±0.013
Trichome	0.03±0.002

For histochemical research fresh free-hand sections were used and were stained as follows: Ponceau 2R plus Azure II for a differential staining of storage proteins (Gutmann, 1995); Alcian Blue (Pearse, 1985) for polysaccharides. Specific staining procedures comprised ferric chloride for dihydroxyphenols (Guerin et al., 1971).

Fresh material was also used for fluorescence analyses using a Leica DM LS fluorescence microscope with a 13 BLU 450–490 nm filter.

3. Results

The leaves of *R. officinalis* are covered with a thick cuticle. A uni-layered epidermis is present on the abaxial and adaxial surface. The leaves bear non-glandular and glandular trichomes on both sides of the lamina. Non-glandular trichomes are present on the veins and leaf margins. Some of the non-glandular trichomes were unbranched but others, more numerous, were multicellular and branched (Fig. 1A, B).

The following types of the glandular trichomes can be recognized: peltate and two types of capitate trichomes.

The peltate trichomes were predominantly on the abaxial surface and located in epidermal depressions. They consisted of one basal epidermal cell, a wide unicellular stalk cell and a multicellular secretory head (Fig. 1C).

The capitate trichomes were situated on the adaxial and abaxial leaf surface, and they were more numerous than peltate trichomes. Capitate trichomes (type I) consisted of a basal cell, a short unicellular stalk and a rather large one- or two-celled secretory head (Fig. 1D). Capitate trichomes type II consisted of a basal cell, a short mono- or bicellular stalk and unicellular secretory head (Fig. 1E).

3.1. Stereology

The values obtained for the volume density of the leaf structures are presented (Table 1). The volume density of

Table 2

Volume density (mm⁰) and number of glandular hairs (1 mm²) of *Rosmarinus officinalis* leaves (mean±S.E.M.)

Trichome type	Volume density (mm ⁰)	Number of hairs/mm ²
Peltate	0.016±0.0017	7.21±0.55
Capitate type I	0.005±0.0007	9.64±0.71
Capitate type II	0.009±0.0007	1.94±0.15

Table 3
Histochemistry of the secretion of the glandular trichomes of *Rosmarinus officinalis* leaves

Staining procedure	Target compounds	Observed colour	Peltate trichomes	Capitate type I	Capitate type II
Ponceau 2R plus Azure II	Proteins	Dark red	+	+	+
Alcian Blue	Polysaccharides	Blue-violet	+	+	+
Ferric chloride	O-dihydroxy phenols	Dark brown	+	–	–

adaxial epidermis was higher than that of abaxial epidermis, whereas the volume density of mesophyll was the highest compared to the central cylinder and intercellular spaces. The values obtained for the volume density and number of morphologically distinct types of glandular trichomes of rosemary are presented (Table 2). The volume density of peltate trichomes was the highest, compared to capitate trichomes types I and II. The values obtained for number of these trichomes

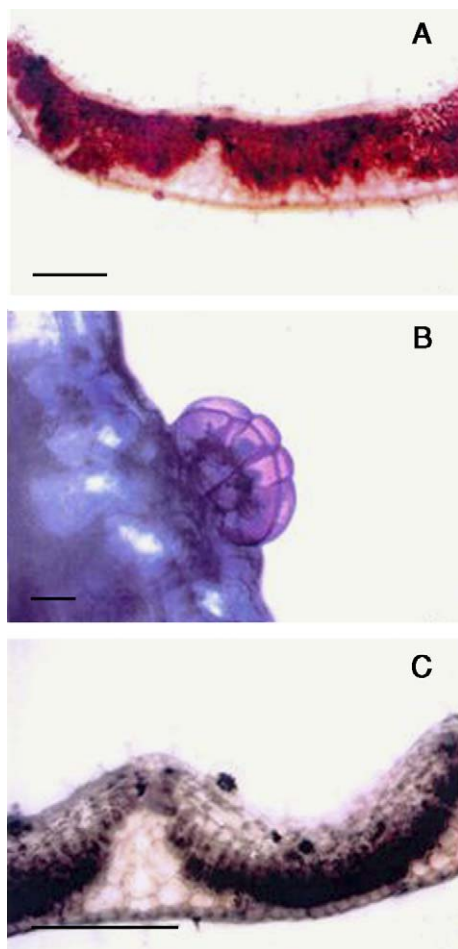


Fig. 2. Histochemical characterization of the secretions of *Rosmarinus officinalis* leaf glandular trichomes: (A) secretion stained dark red with Ponceau 2R plus Azure II, bar=1000 µm; (B) blue-violet staining of secretion with Alcian Blue, bar=100 µm; (C) FeCl₃ test showing dark-brown staining of secretory material, bar=1000 µm.

showed that the capitate trichomes type II were the most abundant.

3.2. Histochemistry

The histochemistry of the secreted products of the glandular trichomes is presented (Table 3). For the histochemical analysis of the secreted material, several staining methods were used. With the Ponceau 2R plus Azure II procedure (Fig. 2A) the reaction was positive and stained dark red both in the peltate and capitate trichomes indicating protein compounds. Staining blue-violet with Alcian Blue for polysaccharides, gave a positive reaction in the secreted material of all glandular hairs especially of head cells of peltate trichomes (Fig. 2B).

Phenolic substances, detected by ferric chloride and stained dark brown, were found in peltate hairs, particularly in stalk cells (Fig. 2C).

3.3. Fluorescence microscopy

Greenish-yellow autofluorescence on the surface of the leaves and on non-glandular trichomes indicated the presence of suberin or cutin-like hydrophobic substances (Fig. 3A). Phenolic substances showed red autofluorescence in the head of peltate trichomes (Fig. 3B). Capitate glandular trichomes showed bright yellow autofluorescence of the secreted material (mainly hydrophilic compounds) inside the head cell (Fig. 3C).

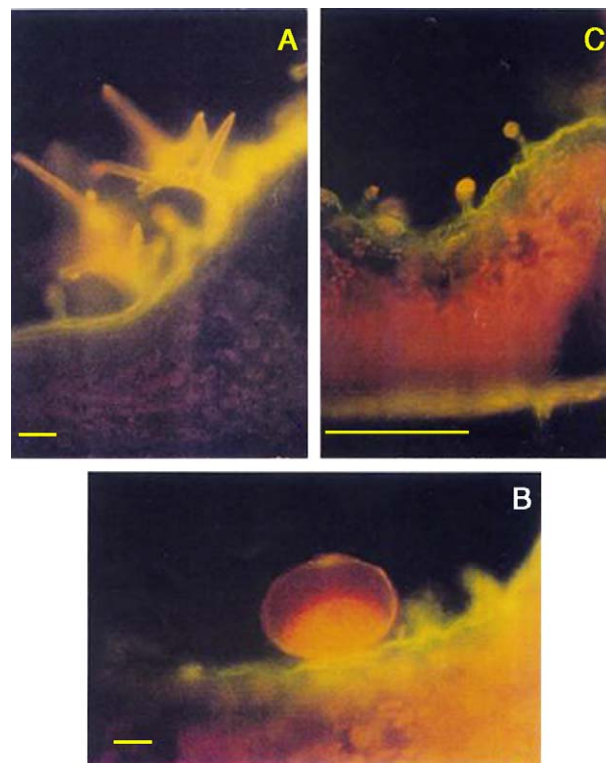


Fig. 3. Autofluorescence of secreted materials of glandular trichomes of *Rosmarinus officinalis* leaves: (A) greenish-yellow autofluorescence on non-glandular trichome, bar=100 µm; (B) red autofluorescence in the head of peltate trichomes, bar=100 µm; (C) bright yellow autofluorescence in the head of capitate trichomes, bar=1000 µm.

4. Discussion

A great variety of important chemical compounds are produced by specialized secretory cells, on many plants in the form of trichomes, and have various functions in the plants themselves. Some of the secreted substances, for instance, serve to attract pollinators and vectors for seed dispersal; others deter phytophagous animals (Fahn, 2000).

The non-glandular trichomes are diverse in morphology, anatomy and microstructure. Basically, they are classified according to their morphology. They may be unicellular or multicellular, and both types can be unbranched and branched. The variability in coverage, by both non-glandular and glandular trichomes and different proportions of these types, between species and even between the two sides of organs, such as leaves, bracts, sepals and petals, is observed. They may appear on one side only or on both sides, equally or unequally (Bosabalidis and Skoula, 1998).

In our investigation of *R. officinalis*, numerous non-glandular trichomes, especially multicellular and branched, were observed, which was also described by Werker et al. (1985a).

The glandular trichomes vary in the following ways: in the chemical composition of the substances they secrete, in their mode of production, in their structure, location and function. All these differences serve at some level, often overlapping, in classification (Werker, 2000).

As in most Lamiaceae species, the surface of *R. officinalis* leaves and calyx possess two types of glandular trichomes: peltate and capitate (Bottega and Corsi, 2000). On the abaxial surface, the capitate glandular trichomes were densely distributed while the peltate ones are scattered among them. Non-glandular trichomes were abundant on the leaf margins. In the present study, we have found only two types of capitate trichomes (type I and type II), different from the results obtained by Werker et al. (1985a), where three types of capitate trichomes were noticed.

The application of unbiased stereological methods to the study of plant anatomical structure can lead to the estimation of many important structural parameters at the same time, such as the mesophyll volume, the proportion of intercellular spaces in the leaf, the mesophyll surface area, the number of mesophyll cells in the leaf, and the mean mesophyll cell volume (Kubinova, 1991).

The result obtained in present study for the volume density of the leaf structures is the first report for *R. officinalis*. Beside the estimation of volume density and number of morphologically distinct types of glandular trichomes on the leaf surfaces, the analysis of the volume density of epidermis, mesophyll, central cylinder and intercellular spaces was done. Stereological investigation showed that the volume density of mesophyll as well as mechanical tissues were high compared to other structures. The volume density of peltate trichomes was the highest, compared to capitate trichomes, and the capitate trichomes type I were the most abundant.

The data from histochemical tests revealed that the secreted material in all types of *R. officinalis* trichomes were of a complex nature. The histochemical results, obtained with

Ponceau 2R plus Azure II, Alcian Blue and Ferric chloride, indicated that glandular hairs produce a heterogeneous secretion, containing proteins, phenolic compounds and polysaccharides. The phenolic compounds are, among others, known to possess antimicrobial properties (Pahlow, 1989). Strong positive reactions with Alcian Blue and Ponceau 2R plus Azure II were especially visible in head cells of peltate trichomes, while positive reaction of Ferric chloride was detected in stalk cells of peltate trichomes.

These results are consistent with the phytochemical data obtained from other analyses of the Lamiaceae (Richardson, 1992; Ascensão and Pais, 1998). The autofluorescence observed is also noted by other authors (Vermeer and Peterson, 1979). The main secretion of peltate trichomes of rosemary that shows autofluorescence is composed of proteins, polysaccharides and *O*-dihydroxyphenols. The results regarding structural characteristics of glandular trichomes and histochemical analysis could be applied in the studies of their medicinal properties. The specific function of each trichome type found in rosemary is not known, and more detailed anatomical, ultrastructural and physiological studies need to be done.

Acknowledgements

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