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Do Non-Aromatic Labiatae Produce Essential Oil? The Case Study of *Prasium majus* L.

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Prasium majus L. (Labiatae, Lamioideae) is considered a typical non-aromatic plant. In this work we examined the glandular trichomes present on leaves and inflorescences and the essential oils of plants growing along the Tuscan coast of Italy. The micromorphological study evidenced different types of trichomes responsible for the essential oil production. The essential oil compositions of leaves and flowers were analyzed by GC/MS and are here reported.

Keywords: *Prasium majus,* Labiatae, Lamioideae, glandular trichomes, micromorphology, histochemistry, essential oil, GC/MS analysis.

The Labiatae family contains about 200 genera of which 40 per cent possess aromatic properties. Owing to these features, the family has a wide economic importance and several species have been widely studied. Currently the family is divided into seven different subfamilies [1], among which the largest ones are Nepetoideae and Lamioideae. Aromatic plants are mostly included within Nepetoideae [2], while species belonging to Lamioideae are usually non-aromatic plants, showing either scarce or absent essential oil production [2].

The production of essential oil is associated with the presence of highly specialized secretory structures known as glandular trichomes. Two types of glandular trichomes are recognized: peltate and capitate [3,4]. Peltate hairs are considered the site of synthesis and storage of essential oil [5], while capitate hairs present a more complex hydrophilic secretion, in which mucopolysaccharides prevail [4,5].

Prasium majus L. (Lamioideae) is considered a typical non-aromatic plant, lacking peltate hairs and showing only capitate hairs [3,5].

This species is an evergreen perennial shrub with white to pale lilac flowers, and leaves with a distinct glossy green colour [6]; neither leaves nor flowers give off any scent. The plant grows in maquis, guarigue, among bushes or rocks, field boundaries and beside dry-stone walls, mainly facing the sea. This plant is widespread in the whole Mediterranean basin and in central and southern Portugal [7]; in Italy, in particular, it is found in southern regions and Sardinia and the northern limit of its distribution area is the Tuscan Archipelago [8]. Concerning the micromorphology, only the anatomy of nutlets has been investigated [9].

Few popular uses have been reported for this plant; it has been employed medicinally in Greece as a tranquilizer [10], and in Tunisia its leaves are used in popular medicine for their soothing properties [11]. The plant is also consumed as a raw food in Tunisia [11], in Crete it is stir-fried or used in traditional vegetable pies [12], and in Sicily (Palermo and Trapani provinces) its use is limited to rural communities [13].

Some non-volatile products have been isolated and identified in the plant [10,14,15]. Recently the

Table 1: Distribution of the different types of glandular trichomes present on vegetative and reproductive organs of *P. majus*. Results: (-) absent; (±) scarce; (+) present; (++) abundant.

	Stem	Leaf/ Bract		Calyx		Corolla	
		adax	abax	adax	abax	adax	abax
A	-	-	-	-	+	-	-
B	+	+	+	-	+	-	±
C	-	-	-	-	++	-	+

essential oil composition of plants from Greece was reported [16].

In this current study we examined specimens of *P. majus* collected from along the Tuscan coast (Italy), during the blooming period (April-June), in order to describe the glandular trichomes present on leaves and flowers and to determine their type of secretion, particularly of essential oil. The essential oils both from leaves and inflorescences were obtained by hydrodistillation and their compositions were determined by GC/MS analysis.

Micromorphological analysis

The glandular trichomes included peltate (type A) and capitate types (types B and C) (Figure 1). Stem and leaves bear only few (Table 1) short capitate trichomes (type B), widely diffused and described in the whole Labiatae family [4,5] (Figure 1). They consist of one basal epidermal cell, one stalk cell and a secretory head (25-30 μm in size) of four cells, with a small subcuticular space in which the secretion is temporarily stored.

Histochemical staining (Table 2) indicated secretion of polysaccharides (Figure 2) and a small amount of essential oil.

The secreting cells ultrastructure shows numerous Golgi bodies and an abundant rough endoplasmic reticulum (RER), involved in polysaccharidic secretion [17], and few electron dense plastids responsible for essential oil production [17] (Figure 3).

On the inflorescences, especially on the abaxial surfaces of the calyx (Figure 1) and corolla, besides the described type B trichomes, other types of glandular hairs are observed: several type A and numerous type C (Table 1). Moreover, short uniseriate non-glandular hairs (type D) are present (Figure 1).

Type A trichomes, unlike the typical peltate hairs, present an elongated basal epidermal cell which

Table 2: Histochemical tests on the different types of glandular trichomes. Results: (-) negative; (±) scarce; (+) intense; (++) very intense.

Staining	Target compounds	A	B	C
Nile Red	Neutral lipids	+	±	++
Fluoral Yellow	Total lipids	+	±	++
NADI reagent	Terpenes	++	±	+
Ruthenium Red	Polysaccharides	-	+	+
Alcian Blue	Polysaccharides	-	+	±
$FeCl_3$	Polyphenols	-	-	+

forms a well developed stalk, so that these trichomes are raised on the epidermal surface (Figures 1 and 4). This uncommon feature was already observed in *Salvia officinalis* [18] and in several species of *Stachys* [19]. The neck cell, the broad glandular head (40-50 μm in size) of eight secreting cells and the large subcuticular space present the typical morphology quoted in the literature [4,5,17]. The secretion stored in the subcuticular space is composed of essential oil (Table 2), since it shows a strong positive reaction only to the Nadi reagent (Figure 4). The most striking ultrastructural features observed in the cytoplasm of the secreting cells are plastids with large starch granules (Figure 5), associated with smooth endoplasmic reticulum (SER). These cellular compartments are typically involved in essential oil production and transfer [17].

Type C long capitate trichomes (Table 1; Figure 1), observed also in several *Stachys* species [19], consist of one basal epidermal cell, a stalk of two-three cells and a multicellular head (40-60 μm in size) of six-eight cells. Each glandular cell is endowed with a small subcuticular space; the secretion is extruded to the outside from the subcuticular space and also from the whole external wall and flows along the stalk to the epidermis [19]. The secretion shows a complex composition (Table 2), since it contains polysaccharides, essential oil and polyphenols (Figures 6 and 7).

In young trichomes, the glandular cells ultrastructure shows mitocondria, Golgi bodies, RER vesicles and multi-shaped plastids with starch granules (Figure 8). In mature trichomes, Golgi bodies and RER elements occur occasionally, while plastids, SER and lipidic droplets (Figure 9) can be observed. Therefore, these hairs present different types of secretion according to the different ages of the trichomes.

Essential oils analysis

Very small amounts of essential oils were obtained by hydrodistillation of the leaves and inflorescences; their compositions are reported in Table 3.

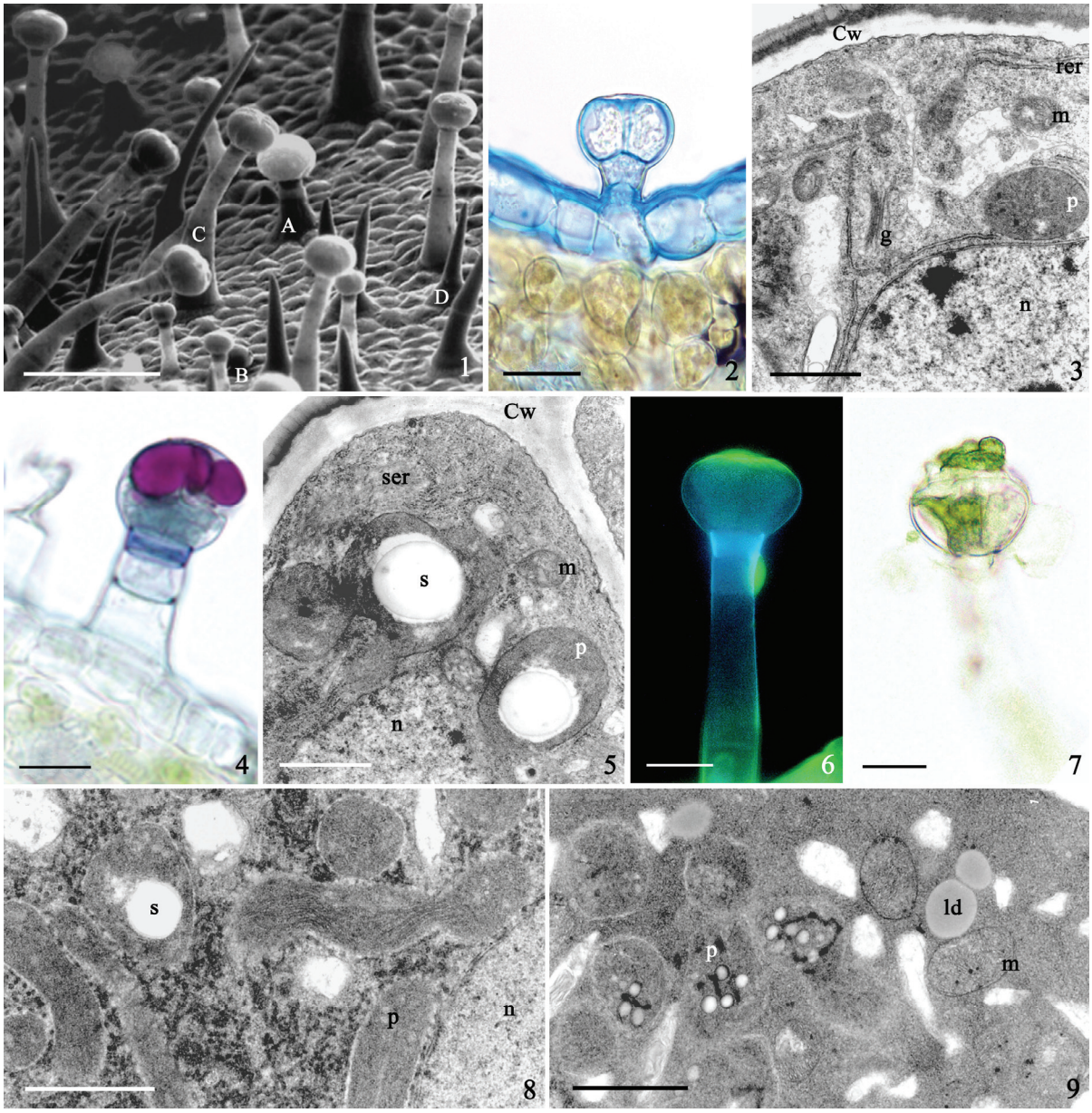

Figure 1: Trichomes on the abaxial side of the calyx: A. peltate, B. short capitate, C. long capitate and D. simple uniseriate non-glandular trichomes. Bar = 100 µm. **Figure 2**: Histochemistry of type B trichome: Alcian Blue. Bar = 25 µm. **Figure 3**: Secreting cell cytoplasm of type B trichome. Bar = 1 µm. **Figure 4**: Histochemistry of type A trichomes: Nadi reagent. Bar = 25 µm. **Figure 5**: Secreting cell cytoplasm of type A trichome. Bar = 1 µm. **Figures 6**, **7**: Histochemistry of type C trichome: Fluoral Yellow 088 (6) and $FeCl_3$ (7). Bars = 25 µm. **Figures 8, 9**: Secreting cell cytoplasms of a young (8) and mature (9) type C trichome. Bars = 1 µm. (Cw) Cell wall; (g) Golgi bodies; (ld) lipidic droplets; (m) mitocondria; (n) nucleus; (p) plastid; (rer) RER; (s) starch; (ser) SER.

The essential oil compositions of flowers and leaves differ. The volatile compounds of leaves are characterised by phytol (35.5%), (*E*)-caryophyllene (19.1%), and hexadecane (7.0%). Oxygenated diterpene hydrocarbons (35.5%), sesquiterpene hydrocarbons (25.0%), and hydrocarbons (22.1%) are the principal fractions. The essential oil of flowers is characterised by six main compounds, (*E*)-caryophyllene (43.6%), 6,10,14-trimethyl-2-pentadecanone (7.5%), 9,12-octadecadienal (5.9%), germacrene D (5.7%), pentadecane (5.7%), and tetradecane (5.6%). Sesquiterpene hydrocarbons (56.9%) and hydrocarbons (19.7%) are the principal fractions.

In conclusion, *P. majus*, considered a typical non-aromatic plant [3,5], bears glandular trichomes which produce small quantities of essential oils, both in leaves and flowers. The plant presents few glandular trichomes on its leaves (type B), but numerous on its inflorescences (types A, B and C). Histochemical observations indicate that the three

Table 3: Essential oil compositions of leaves and inflorescences of *P. majus*. RI = Retention Index.

Compounds	RI	Leaves %	Flowers %
α-Pinene	938	0.1	-
β-Pinene	981	0.1	-
o-Cymene	1026	0.3	-
γ-Terpinene	1060	0.2	-
Thymol	1293	2.7	-
α-Terpinyl acetate	1353	0.5	-
α-Ylangene	1377	1.7	3.7
Tetradecane	1400	2.7	5.6
(*E*)-Caryophyllene	1418	19.1	43.6
Germacrene D	1483	2.5	5.7
Pentadecane	1500	1.4	5.7
δ-Amorphene	1512	1.7	3.9
Caryophyllene oxide	1580	-	1.4
Hexadecane	1600	7.0	4.0
9,12-Octadecadienal	1645	-	5.9
Heptadecane	1700	0.9	2.3
6,10,14-Trimethyl-2-pentadecanone	1791	-	7.5
Octadecane	1800	2.6	1.0
Hexadecanol	1875	-	2.1
Nonadecane	1900	0.2	-
Methyl hexadecanoate	1920	0.1	-
Hexadecyl acetate	2001	1.5	-
Octadecanol	2074	5.1	-
Heneicosane	2100	1.9	1.1
Phytol	2112	35.5	3.5
Docosane	2200	0.5	-
Tricosane	2300	0.5	-
Tetracosane	2400	1.0	-
Pentacosane	2500	3.5	-
Total		**93.3%**	**97.0%**

types of trichomes produce different kinds of substances (polysaccharides, phenols and essential oil). The organelles observed in the cytoplasm of the secreting cells are consistent with these types of secretion.

Essential oil of leaves is produced by type B trichomes, the only type present, considered a typical mucopolysaccharides producer [4]. In this species they are responsible also for the production of the terpenoid fraction, as already observed in *Stachys recta* [20].

The inflorescences, besides type B hairs, bear other types of trichomes, already described for the Labiatae [18,19]. Type A trichomes have a typical essential oil secretion, while type C trichomes produce a complex secretion, which contains both hydrophilic and lipophilic substances.

Essential oil was obtained and analyzed also in flowering plants from Greece [16], but the yield of essential oil is not reported. The composition of our samples differs from those of Greece: only α-pinene, γ-terpinene, thymol, (*E*)-caryophyllene, caryophyllene oxide, and tricosane are present in plants from both sites. Samples A and B from Greece are characterized, respectively, by 1-octen-3-ol (20.7%) and dehydro-aromadendrene (31.8%).

The differences could be ascribed not only to the different plant material examined (fresh leaves and flowers in our work, the whole dry plant at flowering time in Greek samples). However, the essential oil composition is certainly affected by the different origin of plant material: the northern part of the distribution area for our samples and typical Mediterranean distribution area for Greek samples. Therefore, the different environmental conditions could be responsible for different chemotypes. It would also be interesting to verify if the plants of southern and warmer regions are richer in essential oil than those of the northern regions.

Experimental

Plant material: Specimens were collected from two different localities in Tuscany during the blooming period: 03.05.2005 Baratti (Livorno) and 12.05.2007 Giglio Island, Campese (Grosseto). They were determined according to Pignatti [8].

Micromorphological analyses were performed on fresh material (stems, leaves, bracts, calyces and corollas) using scanning electron microscopy (SEM), light microscopy (LM) and transmission electron microscopy (TEM).

SEM observations: Small pieces of plant material were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 6.8, dehydrated in ethanol in ascending grades up to absolute and then dried using a critical point dryer apparatus. The samples, coated with gold, were observed with a Philips XL-20 SEM.

LM observations: Fresh material was frozen, sectioned and stained using different techniques in order to evidence the different components of the secretion. The stains employed were: Fluoral Yellow-088 for total lipids [21], Nile Red for neutral lipids [22], Nadi reagent for terpenes [23], Ruthenium Red [24] and Alcian Blue [25] for acid polysaccharides, and Ferric Trichloride for polyphenols [26]. Observations were made with a Leitz DM-RB Fluo optic microscope.

TEM observations: Small pieces of plant material were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 6.8 and post fixed in 2% OsO_4, dehydrated in ethanol in ascending grades up to absolute and embedded in Spurr's resin. Ultra thin sections were stained with uranile acetate and lead citrate. Samples were examined with a Philips EM-300-TEM.

Isolation and identification of the essential oils: Fresh leaves and inflorescences of the specimen collected at Giglio Island were separately steam distilled for 3 h, in a Clevenger-type apparatus. The essential oil obtained was dried over anhydrous sodium sulfate and stored in sealed vials under refrigeration prior to analysis.

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS): The GC analyses were carried out using an Agilent 6890N instrument equipped with HP-WAX and HP-5 capillary columns (30 m x 0.25 mm, 0.25 μm film thickness), working with the following temperature programme: 10 min at 60°C, and subsequently up to 220°C at 5°C/min; injector and detector temperatures, 250°C; carrier gas, helium (1 mL/min); split ratio, 1:20. GC/MS analyses were carried out using an Agilent 5975 GC/MS system operating in the EI mode at 70 eV, using the same columns. The identification of the components was made for both the columns, by comparison of their retention time with respect to *n*-paraffin (C6-C22) internal standards. The mass spectra and Kovats Indices (KI) were compared with those of commercial (NIST 98 and WILEY) and home-made library mass spectra built up from pure compounds and MS literature data.

Area percentages were obtained electronically from the GC-FID response without the use of either an internal standard or correction factors.

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