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Research article/Araştırma makalesi

Comparative systematical, morphological, trichomes and phytochemical characterization of *Teucrium montbretii* subsp. *pamphylicum* and *Teucrium odontites*

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Abstract

Teucrium montbretii Bentham subsp. *pamphylicum* P.H. Davis and *Teucrium odontites* Boissier & Ball are endemic species for Turkey. *T. odontites* is endangered (EN) taxa and could be damaged in the future. The aerial organs of *T. montbretii* subsp. *pamphylicum* and *T. odontites* bearnumerous eglandular and glandular trichomes. Eglandular trichomes are simple, long-multicellular with cuticular micropapillae and glandular hairs are of peltate and capitate types. Hydrodistilled essential oils of *T. montbretii* subsp. *pamphylicum* and *T. odontites* were analysed by GC/MS. Caryophyllene oxide (37.4%), β-caryophyllene (29.1%) and caryophyllenol-II (7.9%) were the main constituent in the oil of *T. montbretii* subsp. *pamphylicum*, cadalene (10.3%), caryophyllene oxide (7.2%) and alloaromadendrene (6.0%) were the main component in the oil of *T. odontites*.

Key words: *Teucrium*, Lamiaceae, morphology, trichomes

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Teucrium montbretii and *Teucrium odontites* (Lamiaceae)'nin karşılaştırmalı sistematik, morfolojik, tüy ve fitokimyasal özellikleri

Özet

Teucrium montbretii Bentham subsp. *pamphylicum* P.H. Davis and *Teucrium odontites* Boissier & Ball Türkiye için endemik türlerdir. *T. odontites* endangered (EN) tehlike kategorisindedir ve gelecekte doğadan yok olabilir. *T. montbretii* ve *T. odontites*'in toprak üstü organları çok sayıda salgı ve örtü tüyleri taşır. Örtü tüyleri basit, papilli kütikulalı çok hücreli, salgı tüyleri peltat ve kapitat tipindedir. *T. montbretii* ve *T. odontites*'in hidrodistilasyonla elde edilen uçucu yağları GC/MS cihazında analiz edilmiştir. Caryophyllene oxide (37.4%), β-caryophyllene (29.1%) ve caryophyllenol-II (7.9%) *T. montbretii*'nin ana bileşenleri, cadalene (10.3%), caryophyllene oxide (7.2%) ve alloaromadendrene (6.0%) ise *T. odontites*'in ana bileşenleri olarak bulunmuştur.

Anahtar kelimeler: *Teucrium*, Lamiaceae, morfoloji, tüy

1. Introduction

Lamiaceae has a cosmopolitan distribution and consists of 236 genera and about 7000 species (Steven, 2001). The species belonging to the *Lamiaceae* family are characterized by the presence of glandular trichome. This epidermal appendice is not only in species having a characteristic scent, but also in species which lack fragrance. The literature concerning these trichomes is very rich as regards to morphology, differentiation, ultrastructure and mode of secretion (Werker, 2000; Fahn, 2000).

Turkey is an important gene center for the family Lamiaceae (Başer, 1993). With their pleasant fragrance, many species of Lamiaceae have been used as herbal teas in Turkey. Many of species are used as raw material in cosmetic industry.

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Teucrium L., with about 100 species, is a large and polymorphic genus distributed in Europe, North Africa, and temperate parts of Asia, but mainly in the Mediterranean region (Bentham, 1835). The genus *Teucrium* is represented in Turkey by 36 species and 48 taxa (Vural et al., 2015). *Teucrium* species have been widely used for their tonic, diaphoretic, antidiabetic, antiseptic, carminative, diuretic, stomachic, stimulant, antipyretic and antihelmintic properties in folk medicine (Kaya et al., 2009; Özkan et al, 2007). The taxonomic value of the indumentum and its importance in systematic and phylogenetic relationships is well known in Lamiaceae (Cantino, 1990). In Flora of Turkey (Duman, 2000; Ekim, 1982). *T. montbretii* subsp. *pamphylicum* (Bentham, 1836) was recorded closely related with *T. odontites* (Boissier, 1859). Both species are endemic to Turkey. These taxa are respectively known as "Sürmeli fatmacıkotu" and "Hamesi" in Turkish. The paper compares morphological, trichomes and chemical features of the two species with a view to confirm their taxonomical status.

2. Materials and methods

2. 1. Plant materials

T. montbretii subsp. *pamphylicum* was collected in July 2002 from three different localities in Antalya, whereas *T. odontites* was collected in June 1995 from two localities (Table 1). Voucher specimens are kept at the Herbarium of the Faculty of Pharmacy of Anadolu University in Eskişehir, Turkey.

Table 1. Localities of specimen

Speimens	Locality	Date	Collector	Altitude	Herbarium
T. montbretii subsp. *pamphylicum*	Antalya: Manavgat, Oymapınar	08/07/2002	S. Küçük 1008	200 m	ESSE: 14412
	Antalya: Kemer, Gedelma-Kesme boğazı	21/06/1995	K.H.C. Başer	35 m	ESSE: 11301
	Antalya: Konyaaltı	21/06/1995	K.H.C. Başer	14 m	ESSE: 11304
T. odontites	Antalya: Kaş-Kalkan road, Kakutaş	20/06/1995	K.H.C. Başer 1045	73 m	ESSE: 11382
	Antalya: Kemer-Çamdağ	21/06/1995	K.H.C. Başer	30 m	ESSE: 11303

2. 2. Morphological studies

Morphological features were determined on herbarium materials and living specimens. Olympus SZX12 Stereomicroscope with drawing tube was used in morphological studies. Detailed species descriptions and diagnostic features are given.

2. 3. Scanning electron microscopy (SEM)

Stems, leaves and calyces were fixed with 3% glutaraldehyde in 0.1M sodium phosphate buffer, pH 7.2, for 4 h at 4 ºC. After washing, the material was dehydrated by acetone critical point, and dried. The specimens were mounted onto SEM stubs using double-sided adhesive tape and coated with gold. Photographs were taken with an electron microscope (Zeiss EVO 50).

2. 4. Light microscopy

Transverse sections and surface preparations of leaves stems and calyces were manually obtained for anatomical observations of glandular and non-glandular trichomes and examined with a Leitz SM-LUX binoculer microscope with drawing tube.

2. 5. Isolation of essential oils

The essential oils from air-dried plant materials were isolated by hydrodistillation for 3 h, using a Clevenger-type apparatus to produce a small amount of essential oil which was trapped in *n*-hexane. The obtained oils were dried over anhydrous sodium sulphate and stored at +4°C in the dark until analysed and tested.

2. 6. GC–MS analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 μm film thickness) was used with helium as carrier gas (0.8 ml/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450.

2. 7. GC analysis

The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

2. 8. Identification of components

Identification of the essential oil components were carried out by comparison of their relative retention times by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, Adams Library, MassFinder 3 Library) (Koenig et al., 2004; McLafferty, 1989) and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data (ESO, 1999; Jennings and Shibamoto, 1980; Joulain and Koenig, 1998) was used for the identification.

3. Results

This study was aimed at comparing morphological, indumentum and chemical features of the two species with a view to establish further proof to their taxonomical identity. Morphological features are shown in Figs. 1–2. The differences between *T. montbretii* subsp. *pamphylicum* and *T. odontites* are summarized in Table 2.

According to our morphological findings, stem length, branching, margin shape, leaf veneation and shape, inflorescence architecture, flower number, bract and bracteol shape, pedicel length, calyx tube and teeth shape. Morphological investigation of the species showed the presence of different hair types on stems, leaves and calyces.

Table 2. Morphological and morphometrical differences of *T. montbretii* subsp. *pamphylicum* and *T. odontites*

Characters		***T. montbretii* subsp. *pamphylicum***		***T. odontites***	
		Flora of Turkey	**This study**	**Flora of Turkey**	**This study**
Plant length		5-40 cm	7-30 cm	10-15 cm	15-25 cm
Stem	**branching**	----	branched	sample or sparsely branched	same
	shape	long and flexuous	same	arcuate-ascending or procumbent	same
	hair type	tomentose-villous, viscidulous	same	spreading-pubescent, hairs shorter than diam. of stem	spreading-pubescent
Petiole	**length**	(2-)4-6 mm	same	---	2-5(-6)mm
Leaves	**measurements of lamina**	---	5-20mm	---	4-13mm
	lamina shape	ovate, ovate-oblong	ovate-deltoid	ovate-orbicular	ovate-rotundate
	lamina base	truncate to rounded	cordate to truncate	rounded or truncate	rounded
	lamina margin	broadly crenate throughout	broadly deeply crenate	crenate-dentate with only c. 3 teeth on each side	crenate-dentate
	apex of lamina	obtuse	rotundate	subacute	same
	hair type on upper face	shortly villosuolus to tomentose	same	sparsely pubescent to subglabrous above, hispidulous below	same

Table 2. continued

	venation	---	camptodromous	---	craspedodromous
Inflorescence	**flower number**	---	20-25	few flowered	10-20
	shape	racemes dense or lax	same	occasionally subpaniculate a base, racemes lax	shorter rasemes
	lenght	5-20 cm.	1-15 cm	---	1-10 cm
Bracts	**measurements of lamina**	---	4-14 mm	subequal to pedicels or shorter	5-12mm
	lamina shape	linear-elliptic to elliptic	broadly ovate	narrowly elliptic	ovate
	lamina base	---	truncate	----	rotundate
	lamina margin	---	distincly crenate	-----	crenate or integer
	lamina apex	acute	rotundate	-----	obtus
Bracteols	**lamina length**	---	2-10 mm		2-8mm
	lamina shape	---	elliptic	---	linear-elliptic
	lamina apex	---	acute	---	subulate
Pedicel	**length**	subequal to calyx or shorter	shorter to calyx	as long as calyces or longer	longer to calyx
Calyx	**length**	3-5 mm	3.5-6 mm	4 mm	4-6 mm
	teeth shape	---	bilabiate 1/4	bilabiate to 1/3	same
	hair type	villous, shortly glandular	same	pubescent	same
Corolla	**colour**	lilac to purplish	same	purple	same
	length	1.5x calyx	5-10 mm	twice as long as calyx	5-10 mm
	hair type on inner face	---	villous	---	glabrous

Three different hair types were observed on vegetative and reproductive organs (Table 3):

A) Peltate Trichomes: peltate glandular hairs with a very short stalk cell and a large secretory head forming the central and peripheral cells. They had 1–8 central and 6–8 peripheral cells. They were present stem, leaves and calyx. (Figs. 3A, 4A and 5–10).

B) Capitate Trichomes: Capitate hairs with a stalk 1–3 celled usually with a short neck cell or no stalk and 1–3 cell head. We observed two types of capitate trichomes: Type I) Capitate trichomes had a round like head cell (Figs., 3B, 4B and 5–10). Type II) Consisting of a cup-like head cell (Figs., 3B, 4B and 5–10).

C) Acicular Trichomes: Acicular hairs, non-secretory multicellular clothing trichomes, unbranced with 1–5 cells, arranged in a single row and have a cuticle with micropapillae (Figs., 3C, 4C and 5–10).

Also, According to our findings essential oils of main constituent are different.

Table 3. Trichomes Distribution on Stems, Leaves and Calyces in species of *Teucrium*

Species	**Plant material**	**A type Peltate trichomes**				**B type Capitate trichomes**				**C type Aciculare trichomes**				
		Centre		**Periphery**		**I Stalk Head**		**II Stalk Head**		1	2	3	4	5
T. montbretii* subsp. *pamphylicum	**Stem**	1	6	0-3	1,2	2,3	1	+	+	+		+		+
	Leaf	1 1	- 6	1-3	1,3	2,3	1	+	+	+		+		+
	Calyx	1	6	1-4	1-3	3	1	+	+	+		+		+
T. odontites	**Stem**	1 1 6	6 8 8	1,3	1,3	2,3	1	+	+	+		+		-
	Leaf	1 6	- 8	1,3	1,3	1,2	1	+	+	+		+		-
	Calyx	1 8	6 8	1	1	3	1	+	+	+		+		-

In Flora of Turkey (Ekim, 1982), mentioned about *T. montbretii* subsp. *pamphylicum* and *T. odontites* are closely related species. Better determine the status of these two taxa for the morphological and morphometric observations on species additions to the Flora of Turkey and some characters have been added to certain differences between the two species have been revealed (Table 2). In the flora, morphological differences between the two species of the shorter indumentum and sharper, fewer indentations are reported (Figure 11). In addition to this difference, according to our results indicated *T. montbretii* subsp. *pamphylicum* stem height 40-50 cm as given in the flora, but as a result of our study it was measured as 14–20 cm and was found to be shorter than *T. odontites*. Leaf length of *T. montbretii* longer than *T. odontites* (5–20 mm), leaf shape ovate-deltoid, leaf base cordate to truncate, margin broadly deeply crenate, apex of lamina rotundate, type of venation camptodromous, number of flowers more than *T. odontites*, bract shape broadly ovate, bract base truncate, margin distincly crenate, apex rotundate, bracteol shape elliptic, apex acute, calyces bilabiate to ¼, while corolla inner surface villous in *T. montbretii* subsp. *pamphylicum*, glabrous in *T. odontites*. The nutlet surface is generally reticulate, but inconspicuous in *T. odontites.* However, *T. montbretii* subsp. *pamphylicum* has flattened nutlets. *Teucrium* sect. *Isotriodon* is characterised by the elongated thick-walled trichomes on the vegetative parts and subsessile glandular trichomes on the nutlets (Navarro and El Oualidi, 2000).

The presence or absence of trichomes and oil glands is also among the most useful taxonomic characters in *Teucrium* L. and can be used as a taxonomic marker in the infrageneric classification of the genus (Dinç et al., 2008; Dinç and Doğu, 2012).

Peltate (A Type) hairs were densely observed on vegetative and reproductive organs, mainly stems, leaves, calyces (Figure 3A-4A, 5–10 and Table 3). While six peripheral cells were observed in *T. montbretii,* eight cells in *T. odontites*. Capitate (B Type) hairs were present stems, leaves and calyces but long capitate hairs densely calyces in *T. montbretii* subsp. *pamphylicum*, sparsely in *T. odontites (*Figure 3B7,8,10 and 4B3,6). Peltate hairs in *T. montbretii* subsp. *pamphylicum* were also much more abundant than in *T. odontites*. In *T. montbretii* subsp. *pamphylicum* and *T. odontites*, acicular hairs are generally abundant. Acicular hairs in *T. montbretii* subsp. *pamphylicum* were 1-5 cells, while in *T. odontites* 5 cell hairs absence (Figure, 3C, 4C and Table 3).

Analyses of the hydrodistilled essential oils were performed on GC and GC/MS systems, simultaneously. The compositions of the oils of *T. montbretii* subsp. *pamphylicum* and *T. odontites* are given in Table 4. According their relative retention indices (RRI) and with their relative percentages (%).

In the oil of *T. montbretii* subsp. *pamphylicum* a sum of forty components were characterized representing 99.4% of the total oil, with caryophyllene oxide (37.4%), β-caryophyllene (29.1%) and caryophyllenol (7.9%) as the main constituents. The essential oil was rich in oxygenated sesquiterpenoids (%55.8).

A total of forty five compounds were characterized in *T. odontites* oil representing 83.2% of the oil. This oil was characterized by a high content of cadalene (10.3%), caryophyllene oxide (7.2%) and alloaromadendrene (6.0%) which was dominated by sesquiterpenoid hydrocarbones (35.3%) and oxygenated sesquiterpenes (33.5%). GC and GC/MS chromatogrammes of the essential oil compositions of these two taxa appeared quite different in terms of chemical composition. More recently, sesquiterpene were found as principal compounds in *T. chamaedrys* subsp. *trapezunticum, T. chamaedrys* subsp. *syspirense, T. capitatum, T. salviastrum* Schreber from Portugal, *T. ramosissimum* Desf from Tunusia, *T. royleanum* Wall ex Benth from Pakistan, and *T. chamaedrys* subsp. *chamaedrys, T. chamaedrys* subsp. *lydium, T. orientale, T. pestalozzae* Boiss, *T. sandrasicum* O. Schwarz, *T. montanum* L. and *T. antitauricum* T. Ekim from Turkey (Kaya et al., 2009).

According to our results, which morphological, indumentum and chemical, these two species is not closely related.

Table 4. The Composition of the Essential Oils of *Teucrium* species

RRI	**Compound**	***T. montbretii* subsp. *pamphylicum*** %	***T. odontites*** %
1017	4-Methyl-2-pentanone	tr	-
1452	1-Octen-3-ol	-	tr
1497	α-Copaene	tr	0.6
1535	β-Bourbonene	0.4	2.6
1553	Linalool	0.6	-
1612	β-Caryophyllene	29.1	1.4
1639	Cadina-3,5-diene	-	1.5
1655	(*E*)-2-Decenal	0.7	-
1661	Alloaromadendrene	0.4	6.0
1687	α-Humulene	3.7	-
1700	Heptadecane	tr	tr
1704	γ-Muurolene	-	3.8

Table 4.continued

1722	Bicyclosesquiphellandrene	-	0.7
1726	Germacrene D	0.5	2.8
1764	*(E)*-2-undecenal	0.3	0.5
1773	δ-Cadinene	0.2	0.8
1776	γ-Cadinene	-	0.4
1849	Calamenene	-	3.5
1868	(*E*)-Geranyl acetone	tr	-
1900	*epi*-Cubebol	tr	0.9
1918	β-Calacorene	-	0.6
1941	α-Calacorene	-	0.5
1945	1,5-Epoxy-salvial(4)14-ene	-	1.5
1957	Cubebol	-	0.9
1973	Dodecanol	-	tr
1984	γ-Calacorene	-	tr
2000	Eicosane	tr	-
2001	Isocaryophyllene oxide	1.4	-
2008	Caryophyllene oxide	37.4	7.2
2037	Salvial-4(14)-en-1-one	-	3.8
2046	Norbourbonone	-	0.2
2057	Ledol	0.8	0.1
2071	Humulene epoxide-II	2.6	0.1
2081	Humulene epoxide-III	0.2	-
2100	Heneicosane	0.1	tr
2130	Salviadienol	-	3.0
2131	Hexahydrofarnesyl acetone	0.5	2.5
2144	Spathulenol	0.2	-
2198	Thymol	0.2	-
2200	Docosane	0.5	-
2210	Copaborneol	-	0.2
2211	Clovenol	0.4	2.2
2239	Carvacrol	0.5	-
2255	α-Cadinol	0.6	-
2256	Cadalene	-	10.3
2300	Tricosane	-	1.4
2316	Caryophylla-2(12),6(13)-dien-5β-ol (=*Caryophylladienol I*)	tr	-
2324	Caryophylla-2(12),6(13)-dien-5α-ol (=*Caryophylladienol II*)	2.0	2.5
2389	Caryophylla-2(12),6-dien-5α-ol (=*Caryophyllenol I*)	2.3	4.4
2392	Caryophylla-2(12),6-dien-5β-ol (=*Caryophyllenol II*)	7.9	4.3
2400	Tetracosane	-	1.0
2445	4-Oxo-calamenene	-	2.2
2500	Pentacosane	0.9	1.4
2503	Dodecanoic acid	0.7	tr
2512	Benzophenone	0.3	-
2600	Hexacosane	tr	0.4
2670	Baeckeol	-	1.9
2679	Manool	1.1	-
2700	Heptacosane	1.9	2.6
2900	Nonacosane	0.3	2.1
2931	Hexadecanoic acid	0.7	0.4
	Oxygenated Monoterpenes	1.3	-
	Sesquiterpene Hydrocarbones	34.3	35.5
	Oxygenated Sesquiterpenes	55.8	33.5
	Diterpenes	1.1	
	Fatty acid+esters	1.4	0.4
	Others	5.5	13.8
	Total	99.4	83.2

RRI Relative retention indices calculated against n-alkanes
% calculated from FID data
tr Trace (< 0.1 %)

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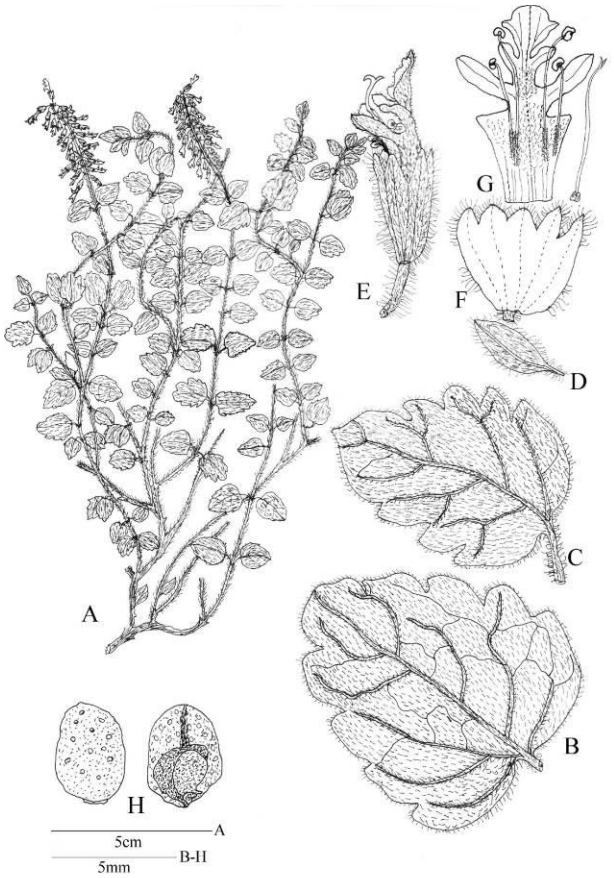


Figure 1. *T. montbretii* (ESSE 14412) A-Habit, B-Leaves, C-Bracts, D-Bracteol E- Flower, F- Calyx, G- Corolla and pistil, H- Nutlets.

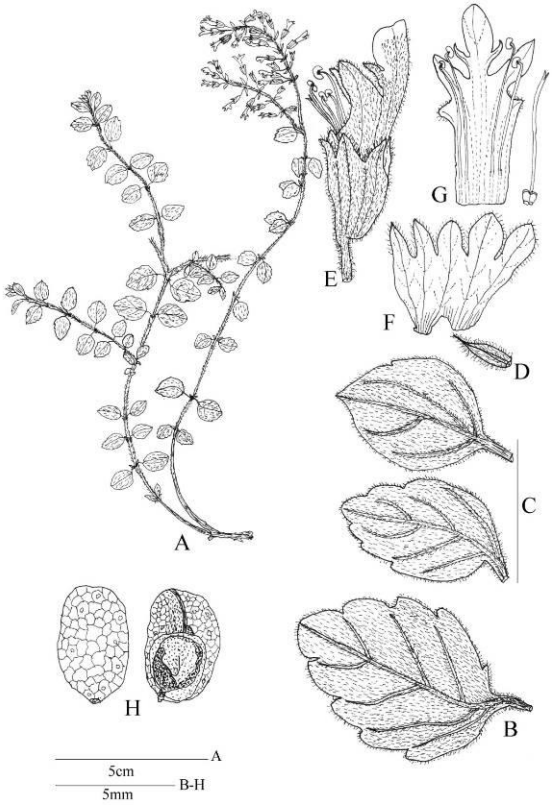


Figure 2. *T. odontites* (ESSE 11382) A-Habit, B-Leaves, C-Bracts, D-Bracteol E- Flower, F- Calyx, G- Corolla and pistil, H- Nutlets.

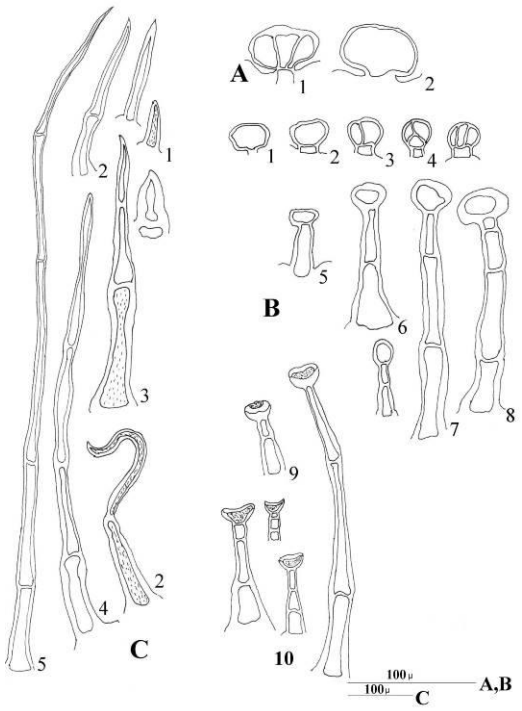


Figure 3. Glandular and non-glandular trichomes of *T.montbretii* in light microscope. A1-2, B1-10, C1-5 type hairs in stem, leaves and calyx.

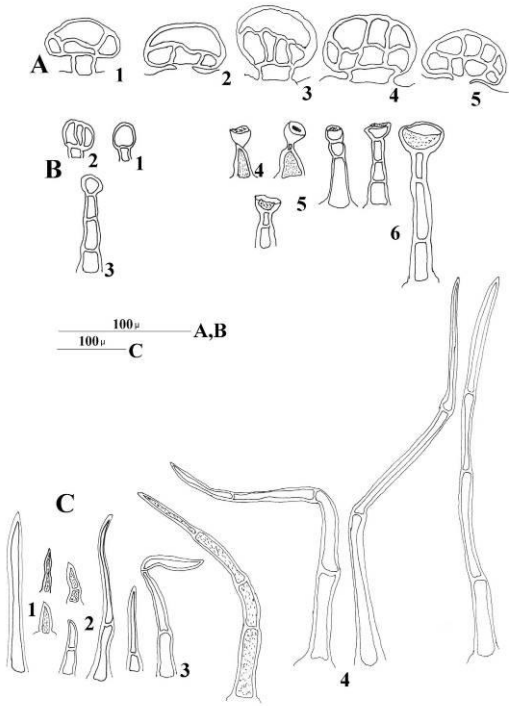
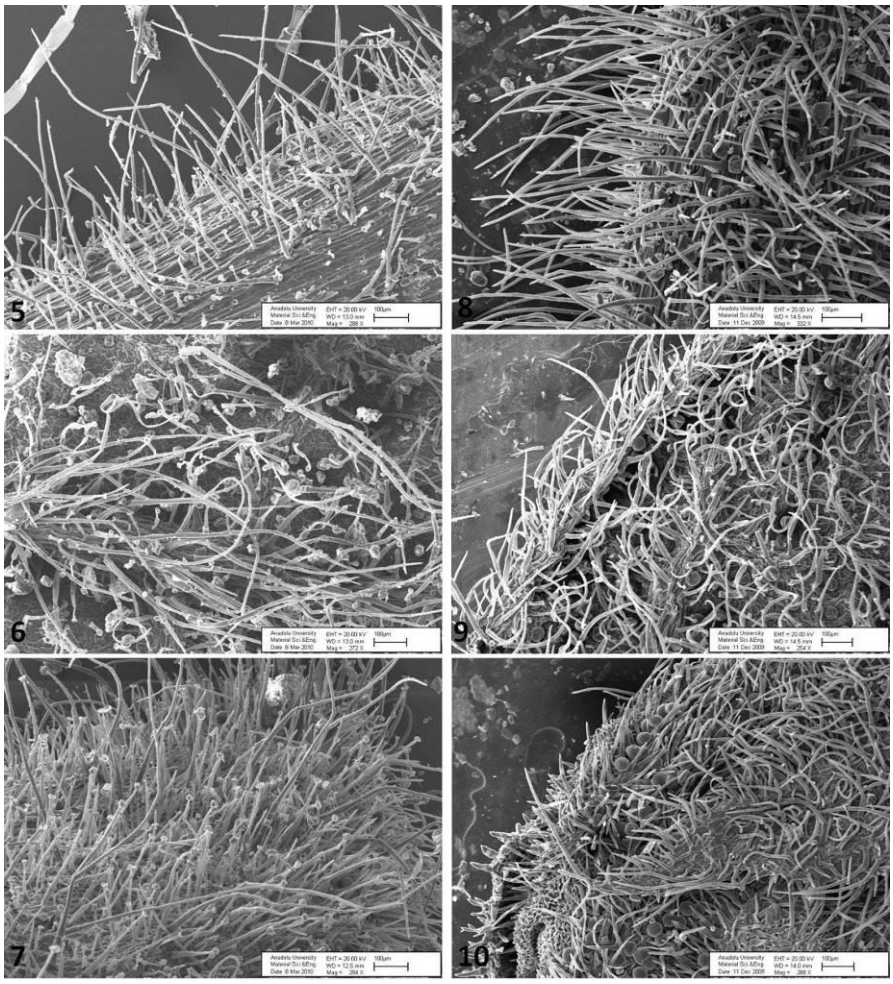


Figure 4. Glandular and non-glandular trichomes of *T. odontites* in light microscope. A1-2, B1-10, C1-5 type hairs in stem, leaves and calyces.



Figs. 5-10. Glandular and non-glandular trichomes of *T. montbretii* A, B, C type hairs stem (5), leaf (6), calyx (7) and *T. odontites* A, B, C type hairs stem (8), leaf (9), calyx (10), in SEM.

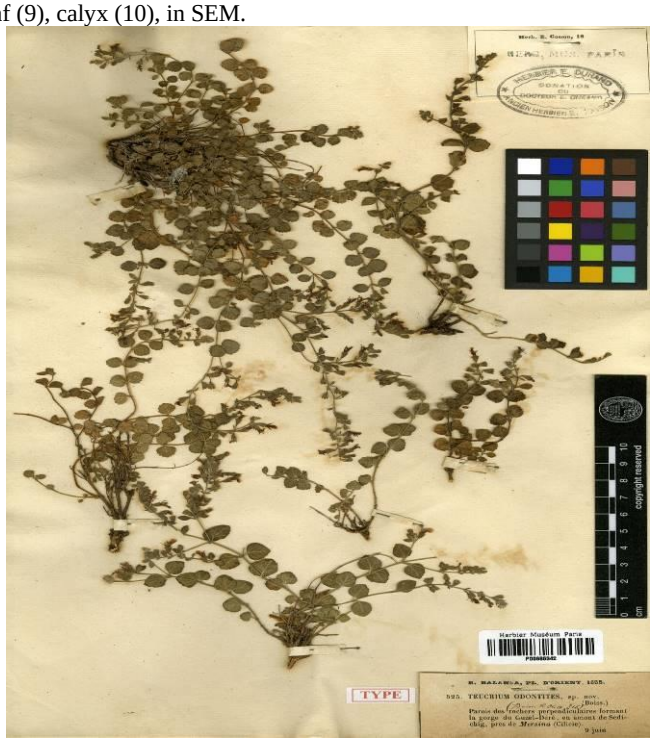


Figure 11. *T. odontites* type specimen

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