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Glandular trichomes and essential oils of *Salvia glutinosa* L.

A Kaya*, B Demirci and KHC Başer

Anadolu University, Faculty of Pharmacy, Pharmaceutical Botany, 26470 Eskisehir, Turkey
** Corresponding author, e-mail: aykaya@anadolu.edu.tr*

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The aerial organs of *Salvia glutinosa* L. bear indumentum with two types of trichomes: simple and multicellular nonglandular trichomes, and stalked and sessile dense glandular trichomes. Glandular trichomes are extremely long-stalked and dense on the stem and calyx surfaces. However, sessile glands are rare on the stem, calyx and leaf adaxial surfaces and dense on the leaf abaxial surface. Secretion accumulates in a subcuticular space and is released to the outside by cuticle rupture. Water distilled essential oil from dried aerial parts of *S. glutinosa* was analysed by GC/MS. The main constituent was identified as 1-octadecanol (11.6%).

Introduction

The essential oil composition of aromatic plants of the family Lamiaceae has been widely studied. The natural essential oils have great commercial value. Little information is available on the morphology, anatomy and development of the glandular structures (trichomes) responsible for secretion of these essential oils (Bosabalidis 1990, Maleci and Servettaz 1991, Maleci *et al*. 1992, Servettaz *et al.* 1992, Özdemir and Şenel 1999, Turner *et al*. 2000).

The genus *Salvia* L. with over 900 species is probably the largest member of the family Lamiaceae and is found in both subtropical and temperate parts of the world (Polunin and Huxley 1967). *Salvia* is represented by 86 species in Turkey (Hedge 1982). Since the most recent revision of the genus, three new species (Davis *et al*. 1988, Vural and Adıgüzel 1996, Dönmez 2001) have been described; the total has now reached 89. Many *Salvia* species are aromatic, rich in essential oils and of potential economic interest besides their ornamental uses. Many of these species are used to flavour food as well as in cosmetics, perfumes and pharmaceutical industries (Marin *et al*. 1996).

Salvia glutinosa L. is an aromatic plant that grows in moist places in deciduous forest and scrub and in Picea forests of north and south Anatolia and the flowering time is from July to October (Hedge 1982). Its aerial organs bear numerous glandular and non-glandular trichomes on their surfaces. The composition of the oil of plants growing in forests in Yugoslavia (Ivanic and Savin 1976) and southern Italy (Senatore *et al*. 1997) has previously been examined.

Previously, we reported the essential oil composition of several *Salvia* species (Başer *et al*. 1993, 1995, 1996, 1997, 1998, Demirci *et al*. in press, Tümen *et al*. 1998). In continuation of our studies on essential oil bearing plants, this paper reports on the morphology and distribution of the glandular trichomes of *S. glutinosa* and the chemical composition of its essential oil.

Material and Methods

Plant material

Salvia glutinosa L. plants were collected during the flowering period (August 2001) from İzmit (Keltepe) province of Turkey. Voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy of Anadolu University in Eskisehir, Turkey (ESSE 13943).

Scanning electron microscopy (SEM)

Leaves, stems and calyces were fixed with 3% glutaraldehyde in 0.1M sodium phosphate buffer, pH 7.2 for 4h at 4°C. After washing the material was dehydrated by acetone critical point drying. The specimens were mounted on to SEM stubs using double-sided adhesive tape and coated with gold. Photographs were taken with electron microscope (Cam Scan S4).

Light microscopy

Transverse sections and surface prepations of leaves stems and calyces were prepared manually for anatomical figures of glandular trichomes and examined with a Leitz SM-LUX binocular microscope with drawing tube.

Gas chromatography Mass spectrometry (GC MS)

The essential oil was analysed using a Hewlett-Packard G1800A GCD system. Innowax FSC column (60m x

0.25mm Ø, with 0.25µm film thickness). Helium (1ml min^{-1}) was used as carrier gas. GC oven temperature was kept at 60°C for 10min and programmed to 220°C at a rate of 4°C min^{-1} and then kept constant at 220°C for 10min then raised to 240°C at a rate of 1°C min^{-1}. Mass range was recorded from *m/z* 35–425. Split ratio was adjusted at 50:1. Injection port temperature was at 250°C. MS were recorded at 70eV. Relative percentage amounts of the separated compounds were calculated automatically from peak areas of the total ion chromatograms. ***n*-**Alkanes were used as reference points in the calculation of relative retention indices (RRI). A library search was carried out using 'Wiley GC/MS Library' and 'TBAM Library of Essential Oil Constituents'.

Results

Types of trichomes observed (Figures 1–7)

A. Heads unicellular
- A1: Short stalked (one basal epidermal cell and 1–3 stalk cells)
- A2: Long stalked (one basal epidermal cell and 4–7 stalk cells)

B. Heads bicellular
- B1: Short stalked (one basal epidermal and one stalk cell)
- B2: Long stalked (one basal epidermal cell and 1–2 stalk cells)

C. Peltate hairs: frequently short stalked (one basal epidermal cell, one stalk cell and four secretory cells or even more)

D. Non-glandular multicellular trichomes, unbranched, uniseriate with cuticular micropapillae

Morphology and distribution of the glandular trichomes

The stems of *S. glutinosa* may rise to c. 1m. They are erect and branched above and are ± densely glandular villous above and hairy below. They have all types of trichomes (A–D) (Table 1). The glandular trichomes are more variable and the A2 type is more frequent on the stem (Figures 1, 2 and 3).

The leaves are simple, ovate-triangular, 8–14cm x 5–11cm, sagittate-hastate, serrate. Leaves of *S. glutinosa* bear glandular (A1, C) and non-glandular trichomes (D) on each site. Short capitate hairs (A1) which are composed of one basal epidermal cell and one stalk cell are more frequent than those with two stalk cells. C hairs are also easily distinguished under the stereoscope. Their heads are four or more celled and secrete essential oil which is formed at the tip of the head between the raised cuticle and the apical cell walls. Since the larger subcuticular space is filled with an apparently foamy secretion (Figures 5, 7), the upper number of the head cell is not determined. Non-glandular trichomes (D) are found mainly on the ribs on the abaxial surfaces. Cell number is up to five on the adaxial surfaces and up to seven on the abaxial surfaces. A2 and B hairs are lacking completely on leaves (Figures 1, 4 and 5).

The calyx is tubular to campanulate, c. 12–17mm in fruit, densely glandular-villous, upper lip 1 dentate, ± straight. The distribution of trichomes on the calyces is particularly important, since the calyx characters are often essential in the taxonomic determination of Lamiaceae. The distribution of trichomes on the outer calyces shows the same kind of hairs as the stems (Table 1). A2 type trichomes are the most common. Small D type trichomes are abundant on the inner surface while the glandular trichomes are absent (Figures 1, 6 and 7).

Trichome distribution on different plant parts in Salvia glutinosa

Trichome distribution on the leaves (adaxial and abaxial surfaces), stem and calyx (inner and outer face) of *S. glutinosa* is shown in Table 1.

Discussion

S. glutinosa bears numerous glandular and non-glandular trichomes. Light (Figure 1) and scanning electron microscopy (Figures 2–7) show details of the outer

Table 1: Trichome distribution on different plant parts in *Salvia glutinosa.* Symbols indicate: – = absence of hairs; ± = few hairs; +, ++ and +++ = increasing presence of hairs.

	Leaves			Calyx	
Hair type	Adaxial	Abaxial	Stem	Inner face	Outer face
A1	+	++	++	–	++
A2	–	–	+++	–	+++
B1	–	–	++	–	++
B2	–	–	+	–	++
C	++	+++	++	–	++
D	++	++[1]	++	+++	±

++[1] mainly on the ribs

A. Heads unicellular
- A1: Short stalked (one basal epidermal cell and 1–3 stalk cell)
- A2: Long stalked (one basal epidermal cell and 4–7 stalk cell)

B. Heads bicellular
- B1: Short stalked (one basal epidermal and stalk cell)
- B2: Long stalked (one basal epidermal cell and 1–2 stalk cell)

C. Peltate hairs: frequently short stalked (one basal epidermal cell, one stalk cell and four secretory cells or even more)

D. Non-glandular multicellular trichomes, unbranched, uniseriate with cuticular micropapillae

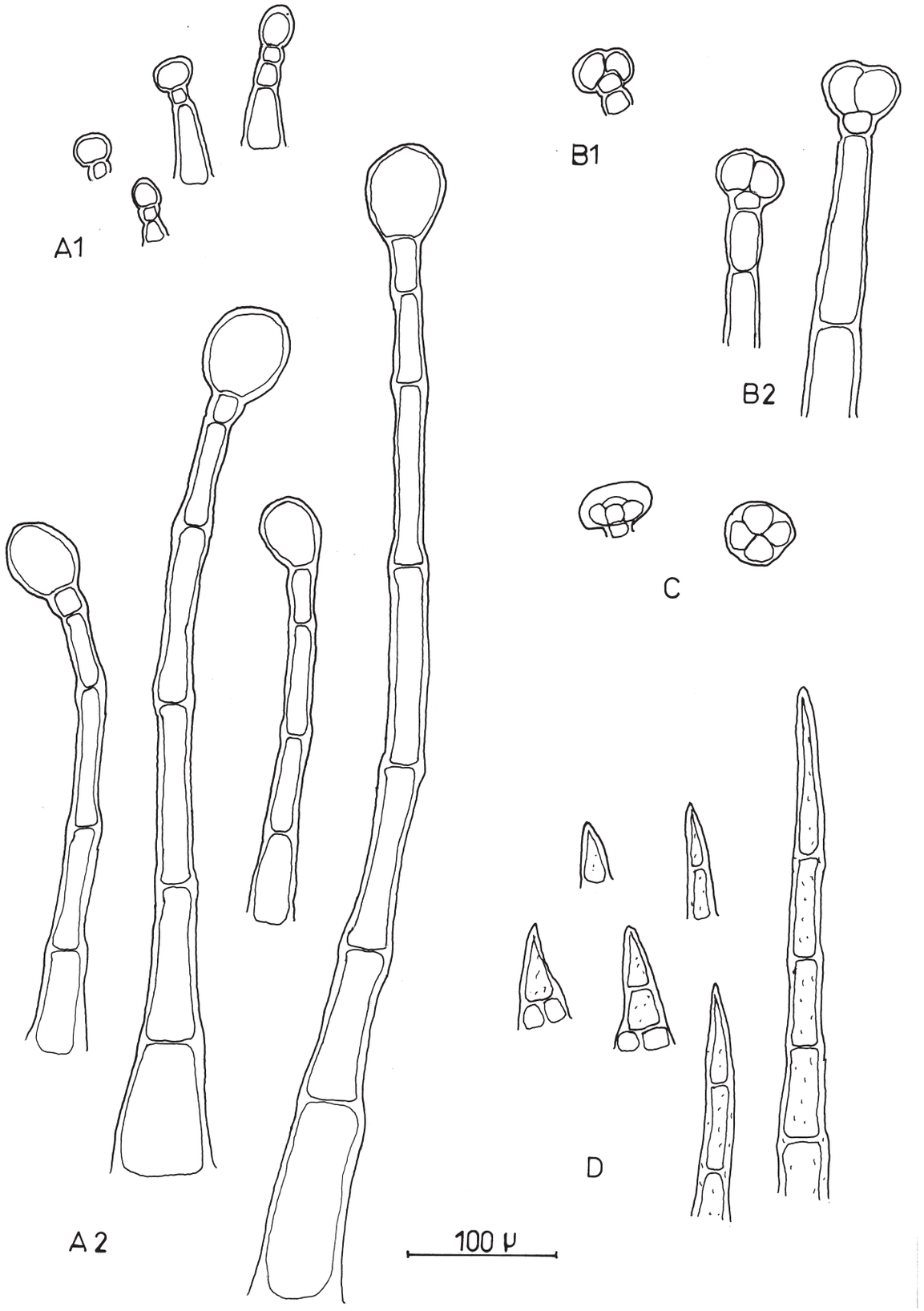
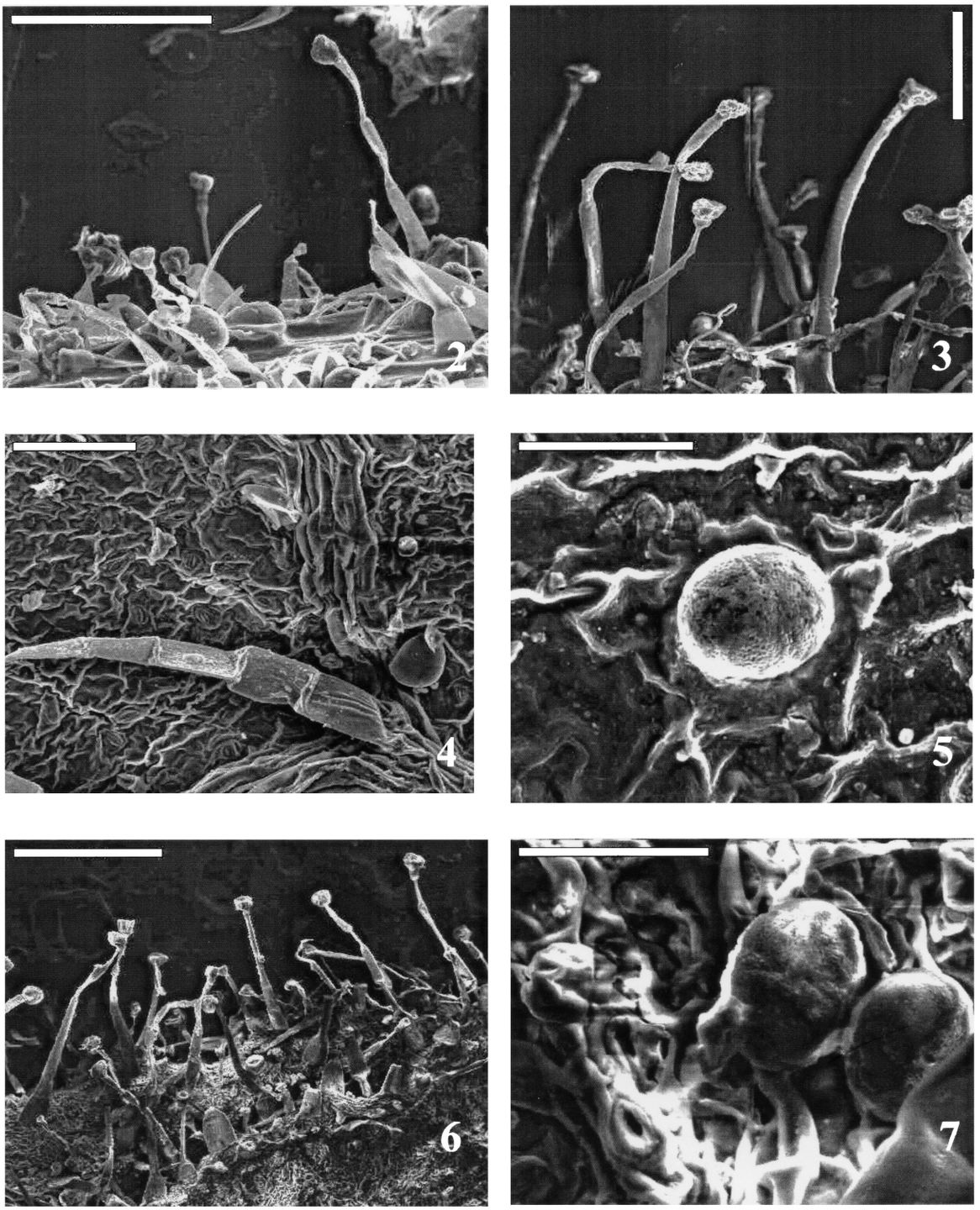


Figure 1: Glandular and non-glandular trichomes of *S. glutinosa* as viewed under a light microscope



Figures 2–7: Glandular and non-glandular trichomes of *S. glutinosa* in SEM. **2–3**: A1, A2, B2, C and D trichomes on the stem. **4–5**: C and D trichomes on the abaxial surface of the leaf. **6–7**: Numerous A2 and C trichomes on the outer surface of the calyx. Scale bars: 2 and 3 = ~200µm, 4 = ~100µm, 5 and 7 = ~50µm, 6 = ~500µm

Table 2: The composition of the essential oil of *Salvia glutinosa*

RRI	Compound	%
1244	Amyl furan (2-*Pentyl furan*)	tr
1304	1-Octen-3-one	tr
1348	6-Methyl-5-hepten-2-one	tr
1360	Hexanol	0.1
1391	(*Z*)-3-Hexenol	tr
1393	3-Octanol	0.4
1400	Nonanal	2.1
1438	Hexyl 2-methyl butyrate	0.3
1452	1-Octen-3-ol	0.7
1466	α-Cubebene	tr
1482	(*Z*)-3-Hexenyl-2-methyl butyrate	0.2
1496	2-Ethyl hexanol	0.1
1497	α-Copaene	2.0
1506	Decanal	0.6
1516	(*E*)-Theaspirane	1.6
1528	α-Bourbonene	0.1
1535	β-Bourbonene	2.9
1548	(*E*)-2-Nonenal	0.1
1553	Linalool	3.0
1553	(*Z*)-Theaspirane	1.2
1571	*trans-p-Menth-2-en-1-ol*	0.2
1590	Bornyl acetate	3.2
1600	β-Elemene	2.3
1612	β-Caryophyllene	4.6
1638	β-Cyclocitral	0.4
1650	γ-Elemene	0.4
1661	Alloaromadendrene	0.1
1663	Phenylacetaldehyde	0.1
1687	α-Humulene	3.5
1693	β-Acoradiene	0.6
1706	α-Terpineol	0.6
1719	Borneol	1.2
1726	Germacrene D	2.1
1741	β-Bisabolene	tr
1758	*cis*-Piperitol	0.7
1766	Decanol	0.1
1773	δ-Cadinene	0.9
1815	2-Tridecanone	tr
1827	(*E,E*)-2,4-Decadienal	0.6
1838	(*E*)-β-Damascenone	1.4
1854	Germacrene-B	3.2
1868	(*E*)-Geranyl acetone	3.9
1880	Benzyl 2-methylbutyrate	tr
1896	Phenyl ethyl isobutyrate	tr
1902	Benzyl isovalerate	0.2
1958	(*E*)-β-Ionone	1.8
1968	1-*endo*-Bourbonanol	tr
1973	Dodecanol	0.8
1988	2-Phenylethyl-2-methylbutyrate	2.3
2008	Caryophyllene oxide	10.7
2037	Salvial-4(14)-en-1-one	tr
2046	Norbourbonone	0.9
2050	(*E*)-Nerolidol	tr
2071	Humulene epoxide-II	3.7
2131	Hexahydrofarnesyl acetone	3.1
2144	Spathulenol	2.0
2179	3,4-Dimethyl-5-pentylidene-2(5H)-furanone	1.7
2214	Phenyl ethyl tiglate	2.3
2300	Tricosane	tr
2384	Hexadecanol	tr
2384	Farnesyl acetone	0.2
2400	Tetracosane	0.1
2500	Pentacosane	0.2
2600	Hexacosane	0.1
2607	1-Octadecanol	11.6
2622	Phytol	1.3
2655	Benzyl benzoate	0.1
2740	Anthracene	0.1
2900	Nonacosane	tr
	Total	88.7

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

morphology of these trichomes. Glandular trichomes of *S. glutinosa* are found in five different forms, i.e. A (A1, A2), B (B1, B2) and C. Cross sections show the anatomy of the glandular trichomes. In such sections, the capitate glandular hairs consist of three cells (a unicellular foot, a unicellular or multicellular stalk and a unicellular or bicellular head). The peltate hairs were similarly observed to be composed of a unicellular foot and a short stalk. Their heads, however, are multicellular (four cells or even more). This pattern of anatomy of the peltate hairs of *S. glutinosa* appears to be similar to that described by Maleci and Servettaz (1991) and Maleci *et al*. (1991, 1992).

On the basis of external morphology the glandular trichomes of *S. glutinosa* are similar to those previously described in Lamiaceae, namely for the leaves of *Satureja thymbra* L. (Bosabalidis 1990) and Italian species of *Teucrium* sect. *Chamaedrys* (Maleci and Servettaz 1991), *Teucrium marum* L., *Teucrium subspinosum* Pourret ex Willd (Servettaz *et al*. 1992) and *Teucrium massiliense* L. (Maleci *et al*. 1992) and *Salvia sclarea* L. (Özdemir and Şenel 1999).

In this study, besides the micromorphological observations, we also report on the analysis of the volatile compounds of *S. glutinosa.* The composition of the essential oils from the aerial parts of *S. glutinosa* is reported in Table 2. Dried aerial parts gave an essential oil yield of 0.1%. 1-Octadecanol (11.6%) was found as the major constituent in the oil.

The essential oil composition of our material was found to be quite different from those already reported. Bornyl acetate was the main component in a sample from Yugoslavia (Ivanic and Savin 1976) while γ-muurolene was found as the major component for the leaves and flowering tops of *S. glutinosa* growing in Italy (Senatore *et al*. 1997). Also the absence of γ-muurolene could be a significant feature of this wild growing species from Turkey while bornyl acetate is represented with 3.2% in our sample.

Various factors, both endogenous and exogenous, can affect the composition of the essential oil of *S. glutinosa.* We believe that the time of flowering, geographical and climatic factors may be very important. Several papers have reported on the variation in the essential oil composition induced by environmental, physiological and edaphic factors which can induce changes in biosynthesis accumulation or metabolism of given compounds of the essential oil (Senatore *et al*. 1997).

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