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

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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 Şenal 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 Adigüzel 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 (Kıltepe) 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 preparations 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 100 cm, 0.25 mm id) was used.
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 cell)
   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. |
|----------------|----------------|----------------|----------------|----------------|
| Hair type | Adaxial | Abaxial | Stem | Calyx |
| A1 | – | – | ++ | – |
| A2 | – | – | +++ | – + |
| B1 | – | – | + | – + |
| B2 | – | – | + | – + |
| C | ++ | +++ | ++ | – + |
| D | ++ | ++ | + | +++ | + |

+++ 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| β-Methylene-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-Cubebeane                                      | tr   |
| 1482| (Z)-3-Hexenyl-2-methyl butyrate                  | 0.2  |
| 1496| 2-Ethyl hexanol                                  | 0.1  |
| 1497| α-Copaene                                        | 2.07 |
| 1506| Decanal                                          | 0.6  |
| 1516| (E)-Theaspirane                                  | 1.6  |
| 1528| α-Bourbonene                                      | 0.1  |
| 1535| β-β-Elemene                                      | 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                                   |      |
| 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-Acromadiene                                    | 0.6  |
| 1706| α-Terpineol                                      | 0.6  |
| 1719| Borneol                                          | 1.2  |
| 1726| Germancrene D                                    | 2.1  |
| 1741| β-Bisabolene                                     | tr   |
| 1758| cis-Piperitol                                    | 0.7  |
| 1796| Decanol                                          | 0.1  |
| 1773| δ-Cadinene                                       | 0.9  |
| 1815| 2-Trimethacane                                   | tr   |
| 1827| (E,E)-2,4-Decadienal                             | 0.6  |
| 1838| (E)-β-Damasconen                                 | 1.4  |
| 1854| Germacrene-B                                     | 3.2  |
| 1868| (E)-Geranyl acetone                              | 3.9  |
| 1896| Benzyl 2-methylbutyrate                          | tr   |
| 1902| Phenyl ethyl isobutyrate                         | tr   |
| 1952| Benzyl isovalerate                               | 0.2  |
| 1958| (E)-β-Ionone                                      | 1.8  |
| 1968| 1-endo-Bourbonan                                 | tr   |
| 1973| Dodecanol                                        | 0.8  |
| 1988| 2-Phenylethyl-2-methylbutyrate                   | 2.3  |
| 2008| Carboxylicene oxide                              | 10.7 |
| 2037| Salvia-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 fagine                              | 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   |

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

The essential oil composition of this wild growing Salvia glutinosa from Turkey is quite different from those previously described in Lamiaiceae, 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 Wild (Servettaz et al. 1992) and *Teucrium massiliense* L. (Maleci et al. 1992) and Salvia scarea 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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