

# Study of Plant Anatomical Characteristics and Essential Oil Composition of Hungarian *Salvia* Species

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## Abstract

We studied the anatomical characteristics, such as the structure and position of glandular and covering trichomes of four *Salvia* species (Lamiaceae): *S. officinalis* L., *S. sclarea* L., *S. pratensis* L. and *S. nemorosa* L. The composition of the essential oil in the various species and their plant organs: leaf, calyx and petal was also investigated. The plants used for this study were cultivated in the garden of the Ecological and Botanical Research Institute of the Hungarian Academy of Sciences. The microscopical studies were carried out using Axioscop apparatus. Extraction of essential oils was carried out by the methods of the Hungarian Pharmacopoea. The essential oil composition was analysed by a gas chromatographic method. Each organ of the *Salvia* species investigated contains glandular and covering trichomes. The labiate-type glandular trichomes are characteristic and other glandular trichomes composed of a unicellular head are also present. On the calyx and petal numerous glandular and covering trichomes can be found. Their structure is similar to those of *S. officinalis*. A few of the glandular trichomes are on the petals, too. A lot of whip-like trichomes are present, but conical unicellular trichomes are rare. The big covering trichomes, having short basal cells and a sharp terminal cell, are characteristic for the leaf of *S. sclarea*. Some conical covering trichomes are also found. The leaf of *S. pratensis* is poor in trichomes. There are rarely multicellular covering and glandular trichomes with a unicellular head. Comparing the essential oil composition of the fresh and dried plant organs of the four *Salvia* species, differences exist only in *S. officinalis*. The dominant components are sesquiterpenes:  $\alpha$ -humulene and caryophyllene in the fresh leaf-oil. In dried leaf oil, the monoterpene thujone isomers were characteristic. The qualitative oil composition of the three organs does not differ, but the percentage occurrence of the components is variable. The thujone isomers are dominant (17-20 %) in the leaf- and calyx-oil, but there is only 5,9 % in the petal-oil. The main component of petal-oil is the pharmacologically active  $\beta$ -pinene (24,4 %). For the composition of *S. pratensis* oil,  $\beta$ -caryophyllene and  $\gamma$ -muurolene are characteristic. Their ratio in the calyx and petal-oils are similar, but in the leaf-oil, this is reversed. The structure of the covering trichomes and the percentage occurrence of the oil components provides a good basis for the differentiation of *Salvia* species.

## INTRODUCTION

The *Salvia* species found in Europe belong to the *Salvia* and *Sclarea* subgenera (Hegnauer, 1969). *Salvia officinalis* L. (cultivated in Hungary), *S. glutinosa* L., and *S. scabiosifolia* Lam. belong to the *Salvia* subgenus. *S. sclarea* L. (cultivated), *S. pratensis* L. and *S. nemorosa* L. (native in Hungary) belong to the *Salvia* subgenus. The species of the *Salvia* subgenus are rich in terpenoids (Bernáth et al, 1991). They contain mono- and sesquiterpenes as constituents of their essential oil, diterpenes in the resin-fraction (bitter substances) and triterpenes, which are mainly found in leaf-cuticle. On the basis of essential oil and resin production significant differences were found between *Salvia* species (Hänsel, 1994). Aromatic species (rich in essential oil) e.g. *S. officinalis* -Species rich in aromatic and resin substances e.g. *S. sclarea* -Species poor in aromatic and resin

substances e.g. *S. pratensis*. Species richest in essential oil are *S. officinalis*, *S. lavandulifolia* and *S. triloba*. Cabo et al. (1987, cit. Hänsel et al. 1994) described four groups on the basis of the main components of the essential oil of *Salvia* species: Species rich in cineol and thujone e.g. *S. officinalis* L.; species rich in camphor and cineol e.g. *S. lavandula* Vahl.; species rich in linalool and linalyl acetate e.g. *S. sclarea* L.; and species containing terpenoids of different types.

The glandular and covering trichomes are the main anatomical characteristics of *Salvia* species. The essential oil and resin are formed in the living glandular hairs; their type is characteristic of the Lamiaceae family. They have one basal cell or a unicellular stem and a head of 4, 8 or 12 cells. On pressing the cuticle surrounding the glandular trichome, the cuticle is opened and the essential oil flows out and the trichome collapses. We can find also some other glandular trichomes with a stalk of several cells and a head of 1-2 cells and with a unicellular stalk and head of 1-2 cells (Hegnauer, 1969). Deufel (1955) described the trichomes of *Salvia* genus in detail. The glandular and covering trichomes were studied by Thoms (1981), Eschrich (1988), Karsten, et al. (1962) and Hänsel et al. (1994). Thoms dealt with trichomes of *Salvia pratensis* (native) and *S. officinalis* L. and *S. sclarea* L. (cultivated in Hungary). The authors mentioned above studied the trichomes of leaves; data concerning other plant organs are not known. On the basis of data found in the literature, the trichomes of *S. officinalis* L. are characterized - beside the labiate- type glandular trichomes - by the trichomes having a head of 1-2 cells and a long shaped whip-like or cone covering trichomes. On *S. sclarea* L., beside the above mentioned characteristics, we found also data for the presence of bristle trichomes of one- or several cell; part of these were shaped like a bill. The trichomes of *S. pratensis* L. are similar to those of *S. sclarea* L. Referring to the anatomical structure of *S. nemorosa* L., there are very few data available. According to Berger (1950), no trichomes occur on the leaves.

## MATERIALS AND METHODS

The plants used for investigation were cultivated in the experimental garden of the Ecological and Botanical Research Institute of the Hungarian Academy of Sciences. The samples are from the collection of 1997-1999.

<i>Salvia officinalis</i> L.	(965/b)	(sage)
<i>Salvia sclarea</i> L.	(8163)	(clary sage)
<i>Salvia pratensis</i> L.	(8725)	(field sage)
<i>Salvia nemorosa</i> L.	(8146)	(grave sage)

We investigated the fresh and dried leaves, calyces and petals. The plant parts were collected in the full flowering period.

The plant organs used for essential oil extraction (dried, fresh) were the following: *Salvia officinalis*, *Salvia sclarea*, *Salvia pratensis* and *Salvia nemorosa* (leaf, calyx, petal). The extraction of essential oils was carried out by the methods of the Ph.Hg.VII. (1992). The plant organs were prepared for the microscopical studies (Axioscop apparatus) as follows. Little pieces of the plant organ (0,5 g) were boiled with 15 ml of water in a porcelain dish till the volume of water was about 7 ml. Then 7-8 ml of ethanolic -potassium chloride was added and boiled for 3-4 minutes. After cooling, 4 ml of 5 % hydrogen peroxide solution was added and boiled again for 3-4 minutes while the plant material was covered with a clarifying top (Sárkány, 1957). The solvent was poured off and the pieces of plant organ were washed with distilled water several times. The essential oil composition was studied by a gas chromatographic method.

## GC parameters

Gas chromatograph FISON GC 8000  
Capillary column: 30 m x 0,32 mm I.D; 0,25 m  
Stationary phase: DB-1701 (OV-17)  
Column temperature: 60°-230°C, 8°C/min, 230°C isotherm 3 min.

Detector: FID, temp.: 240°C  
Carrier gas: nitrogen, pN<sub>2</sub>: 50 kPa, V = 6,8 cm<sup>3</sup>/min.  
Injector temperature: 200°C  
Injection: split: 10 sec (bottom)  
Sample: 0,4 l (l/cm<sup>3</sup> CHCl<sub>3</sub>)  
Evaluation: Chrom Card computer-program

## RESULTS AND DISCUSSION

### Plant-Anatomical Characteristics

On *Salvia officinalis* leaf, covering and glandular trichomes are found. The majority of the glandular trichomes are of the labiate-type with a head composed of 8 cells with a common cuticle. The basal cell of the covering trichomes, built of several cells, is swollen and its end-cell is narrow and whip-like. The basal cells are included in the epiderm-cell and are mobile without braking down. The covering trichomes are bent down on the epidermis and protect the glandular trichomes. The calyces are characterized by labiate-type glandular trichomes and there are also covering trichomes: both of them are whipped, others are cone shaped. Glandular unicellular trichomes are seldom found. A few glandular trichomes are on the petals too. A lot of whip-like trichomes are found, but cone-shaped trichomes with a unicellular head and a stalk of 1-2 cells are rare. The leaf of *S. sclarea* can be characterized by covering trichomes which have a thick base of several cells and a sharp end cell. Some cone-shaped covering trichomes are also found. Besides the labiate-type glandular trichomes, we can find trichomes with a unicellular head and stalk or a stalk of several cells with a unicellular head. Some of the covering trichomes are bristle hairs surrounded by a scale-formation which makes them stiff. On the calyx and petal there are numerous glandular and covering trichomes. Their structure is similar to those of *S. officinalis*.

The leaf of *S. pratensis* is poor in trichomes. There are a few multicellular covering trichomes and glandular trichomes with a unicellular head (Fig.1) The calyx is very rich in trichomes. Glandular trichomes of labiate-type and trichomes with a big unicellular head sitting on a multicellular stalk are also present. The multicellular covering trichomes have a wide basal cell and a pointed end cell. We found also conical unicellular trichomes. On the petal there are less glandular trichomes; most of them have a unicellular head sitting on a multicellular stalk. The covering trichomes are mostly multicellular longshaped.

On the leaves of *S. nemorosa* both glandular and covering trichomes occur. The glandular ones are of the typical labiate-type (Fig. 2). The covering trichomes are multicellular and have a pointed or scaled end, but there are also conical unicellular trichomes. On the calyx we found glandular labiate-type trichomes. The covering trichomes are multicellular, elongated and have a thickened basal cell, but there are some conical shaped trichomes too. For the petals beside the labiate-type trichomes, other glandular trichomes are characteristic which have a multicellular stalk and unicellular head (Fig. 3). The multicellular trichomes are elongated with a deviated end, but we found unicellular trichomes with a parrot-bill shape.

### Essential Oil Composition

Comparing the essential oil composition of the fresh and dried plant organs of the four *Salvia* species there is a difference only in the oils of *S. officinalis*. Sesquiterpenes:  $\alpha$ -humulene and caryophyllene are the dominant components of the fresh leaf-oil, while in the oil of the dried leaf the monoterpene thujone isomers are characteristic. The essential oil composition of different organs of *S. officinalis* is demonstrated in Fig. 4. The qualitative oil composition of the three organs does not differ, but the percentage occurrence of the components is variable. The thujone isomers are dominant (17-20 %) in the leaf- and calyx-oil, but there is only 5,9 % in the petal-oil, where the main component is the pharmacologically active  $\alpha$ -pinene (24,4 %). The main components of the calyx oil

of *S. sclarea* are linalool, linalyl acetate and  $\alpha$ -terpineol, similar to the composition of the commercial clary sage oils. The composition of leaf and petal oils differ. Essentially, they are poorer in linalool and linalyl acetate and the main component is the sesquiterpene  $\beta$ -farnesene. For the composition of *S. pratensis* oil,  $\beta$ -caryophyllene and  $\gamma$ -muurolene are characteristic. Their ratio in the calyx and petal-oils are similar, but in the leaf-oil this is reversed. The oil composition of each *S. nemorosa* organ is the same. The nonidentified constituents are characterized by their retention times: component-1: 6,01 min, -2: 8,08 min, -3: 15,43 min, -4: 16,54 min.

## CONCLUSIONS

Organs of the *Salvia* species studied contain both glandular and covering trichomes. The labiate-type glandular trichomes are abundant and trichomes with a unicellular head are also frequent. The various *Salvia* species can be differentiated on the basis of the structure of the covering trichomes and composition of their essential oils.

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## Figures



Fig. 1. Multicellular trichomes on the leaves of *Salvia pratensis*



Fig. 2. Glandular and covering trichomes on the leaves of *S. nemorosa*



Fig. 3. The unicellular trichomes with parrot-bill shape (*S. nemorosa*).

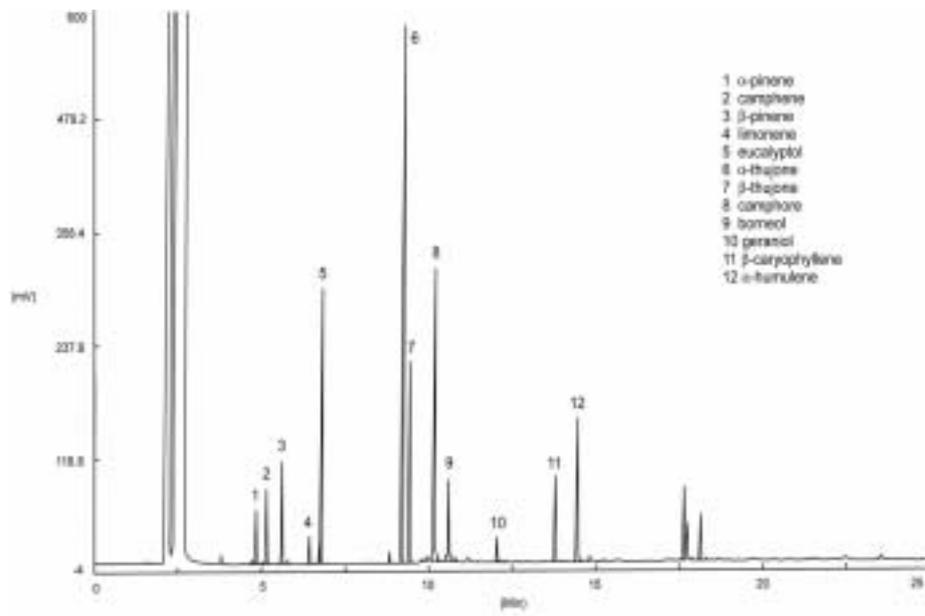


Fig. 4. Essential oil composition of the leaf of *S. officinalis*