Comparative Study on Tannins, Flavonoids, Terpenes and Mineral Elements of some *Salvia* Species

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Abstract

***Salvia officinalis* L., *S. sclarea* L., *S. pratensis* L. and *S. nemorosa* L. originated from Hungary and Transylvania were investigated. Tannin and flavonoid content of the *S.* species were determined and evaluated. Significant differences ($p < 0.05$) were found for tannin content between Hungarian and Transylvanian *S. officinalis* and for flavonoid content between Hungarian and Transylvanian *S. sclarea*. A qualitative comparative study was performed for terpenes from volatile oils by GC and GC-MS technique. The chromatogram of different *S.* species significantly differed from each other. The spectra of the same sage species originated from different places (e.g. *S. officinalis* from Hungary and Transylvania) were not qualitative different. Borneol and α-thujone were detected in each sage species whereas linalyl acetate, α-pinene and β-pinene were found to be present in three sage species. Element concentration of samples was determined by ICP-AES for Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, P, Pb, S, Ti and Zn. The element contrentrations differ significantly between the species and within one species, greatly depend on the place of collection. The common feature of the species examined seems to be the relatively high chromium content. The element content of aqueous extract and the dissolution rate were also determined.**

INTRODUCTION

S. officinalis L. (sage) grows wild in Southern Europe and Asia Minor. In Hungary the plant is cultivated similarly to *S. sclarea* L. (muscat sage). In Hungary several sages grow wild, e.g.: *S. verticillata*, *S. aethiopis*, *S. glutinosa, S. nemorosa, S. pratensis, S. austriaca, S. nutans*. From these *S. nemorosa, S. pratensis* and *S. austriaca* are only known as furrow-weeds and *S. nutans* is a protected plant. In this paper *S. officinalis* L., *S. sclarea* L., *S. pratensis* L. and *S. nemorosa* L. were investigated. The dried leaves of sage (*Salviae officinalis folium*) are registered as a medicinal drug for several indications (inflammatory diseases, common cold, gastric ulcer, state of exhaustion, nervosity) with numerous favourable effects (e.g. antiinflammatory, antifungal, antimicrobial) (Koga et al., 1999, Ozcan and Boyraz, 2000, Baricevic et al., 2001). The food, perfume and pharmaceutical industry use the volatile oil of sage (*Aethorum salviae*) (Then and Konszky, 1987).

Several different investigations have been made on *Salvia* species (Lamiaceae) and it is still in the focus of interest today. The most effective bioactive components of sages are terpenoids formed and stored in Lamiaceae-type grandular hairs. *S. officinalis*. contains mainly monoterpenes (borneol, bornyl acetate, α-pinene, β-pinene, α-thujone, β-thujone, eucalyptol, myrcene) (Bernáth et al., 1991). *S. sclarea* is rich both in mono-terpenes and diterpenes (linalool, sclareol, manool, salvipisone) (Ulubelen et al., 1994/a).

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Di- and triterpenes (B-amyrin, germanicol, lupeol, loranthol) are present in *S. pratensis* (Anaya et al., 1989), while diterpenoids, e.g. nemorosin was found in *S. nemorosa* L. (Ulubelen et al., 1994/b). Each species contains a large amount of flavonoids (Thomas-Barberan and Wollenweber, 1990; Areias et al., 2000) and several papers deal with tanning materials (e.g. caffeic acid, chlorogenic acid, ellagic acid, gallic acid) of sages (Lu et al., 1999; Lu and Foo, 2000). Only some investigations were made to determine the metal ion content of sages (Sullivan et al. 1987; Torshin et al., 1997), although a closer knowledge of the quality and quantity of the metal content would be essential, with special regard to the application of sage in medicine.

For the study we collected different *S. species* from the same site in Hungary and also different *S. species* from the same site in Transyvania. Therefore, the differences between different *S. species* from the same site could be considered as taxonomical variances and the differences between same *S. species* from different site (Hungary and Transylvania) could be geological (or climate) divergences.

MATERIALS AND METHODS

Four *Salvia* species were examined: *S. officinalis* L. (No.:965/b), *S. sclarea* L. (No.:8163), *S. pratensis* L. (No.:8725) and *S. nemorosa* L. (No.:8146). Plants were collected during the flowering period from the botanical garden in the vicinity of Budapest in Vácrátót and from the botanical garden of the University of Medicine, in Tirgu Mures in 2000. For the extraction of the volatile compounds: 10 g of drug was allowed to stand in hexane (100 mL) in room temperature for 5 days and then was filtered. For tea preparation: 2 g of the drug was infused in 200 ml double distilled water. After cooling down, the mixture was filtered. The tannin content of the samples was determined according to the Hungarian Pharmacopoeia (Ph. Hg.VII, 1992) by spectrometry and calculated for pyrogallol as reference material. The total flavonoid content of the samples was determined according to the German Pharmacopoeia (DAB 10, 1996). After acidic hydrolyses of flavonoid glycosides, flavonoid aglycones were complexed with $AlCl_3$, measured at 420 nm and the result were calculated in hyperoside equivalents.

GC measurements of volatile oils were done with a FISON GC800 equipped with a capillar column (30m x 0.32mm, I.D:0.25m) packed with DB-1701 (OV-17). Temperature program: 3 min. at 60 ^oC then heated by 8 ^oC $min.^{-1}$ up to 230 ^oC. GC-MS measurements were made with HP-5890 type instrument. The detection of the components was carried out with Eingine-5989 mass selective detector. Column: 30m x 0.32mm, packed with Supelco PTE-5^{Tm}. Temperature program: 2 min. at 60 ^oC then heated by 10 ^oC $min.^{-1}$ up to 300 ^oC.

The concentrations of elements of samples were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES). Type of instrument: Atom Scan 25 (Thermo Jarrell Ash). *Sampling*: the dry milled samples (0.5 g) or rotavapored extract (20 mL) were digested with a mixture of 5 mL HNO_3 and 3 mL H_2O_2 in teflon vessels. After digestion the samples were diluted to 25 mL, from which the following elements were determined: Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, P, Pb, S, Ti, Zn.

Mean values and standard deviations (SD) were calculated from the results. For comparison of the means one way analysis of variance (ANOVA) was used by GraphPAD software version 1.14 (1990). Significance limit was $p < 0.05$.

RESULTS AND DISCUSSION

Investigations showed that sages originated both from Hungary and Transylvania are rich in tanning materials (Table 1). *S. officinalis* L. shows the highest tannin content followed by *S. sclarea*, *S. pratensis*, then *S. nemorosa*. Almost the same trend can be seen in the flavonoid-, as in the tannin content. *S. officinalis* is the richest in flavonoids and *S. nemorosa* shows the lowest values. Significant difference ($p< 0.05$) was found for tannin content between Hungarian and Transylvanian *S. officinalis* and for flavonoid content

between Hungarian and Transylvanian *S. sclarea*. The most frequently applied method of extraction is making tea. Therefore, the tannin and flavonoid content of extracts are also measured and the results are depicted in Table 1.

The most typical secondary compounds of *Salvia* species are terpenes which were studied by GC. Great differences can be observed in the chromatograms of sage species (Fig. 1) although there was no qualitative difference on the chromatograms of the same sage species originated from different places. *S. officinalis* proved to contain a large amount of monoterpenes from which linalool, α-thujone and α-humulene were identified. The main components of *S. sclarea* are the monoterpenes, linalool and linalyl acetate. The oil of *S. pratensis* contains sesquiterpenes (β-caryophyllene, γ-muurolene) while unknown compounds were found in *S. nemorosa*. The determination of other terpenes was performed by GC-MS. Borneol and α-thujone have been detected in each sage species, and linalyl acetate, β-caryophyllene, α-pinene and β-pinene have been identified in the three sage species, as well (Table 2).

Element concentration of the samples was measured by ICP-AES and significant difference in the element concentration (Al, B, Ba, Cr, Cu, Fe, K, Mg, Mn, Na, P, S, Ti, Zn) of the samples was found by statistical analysis (Table 3). In studying characteristic soil-forming element concentration in the plants, we found the highest Al, Fe, Mn and Ti concentrations in the Hungarian *S. officinalis*. This is probably due to soil pollution of the sample. Zinc is accumulated in the highest amount in the Transylvanian *S. officinalis* and *S. pratensis* and Li content of the Hungarian *S. nemorosa* is also high. Every sample contains Cr and this element, which can be generally detected in the plants in micro concentration, is present in considerable amount in the Hungarian *S. officinalis* and *S. sclarea.* The concentration and the dissolution of elements were also examined (Table 4). Significant differences were found between the element concentrations (Al, B, Ba, Cu, Fe, K, Mg, Mn, Na, P, S, Zn) in different sage teas. The dissolution of elements varied in a wide range according to the sample and element studied.

CONCLUSIONS

A comparative study was made on the phytochemical propertics of four *Salvia* species (*S. officinalis, S. sclarea, S. pratensis, S. nemorosa*) from Hungary and Transylvania. Qualitative comparative investigations were performed for terpenes from volatile oils. Borneol and α-thujone could be detected in each sage species, furthermore linalyl acetate, α-pinene and β-pinene are present in three sage species. The tannin and flavonoid content of the samples differ among the species although generally there is no difference between samples of the same species. Element content of the drug samples and teas highly differs both between species and habitat. In folk medicine, among other medicinal plants, sage is used for curing diabetes mellitus II. (Mueller et al., 1988). The group of compounds responsible for effective therapy is still not known. On the basis of investigations carried out so far, it may be that the common feature of herbs applied in diabetes mellitus is the high Cr content.

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Tables

Table 1. Tannin and flavonoid content in *Salvia* species as determined by spectrophotometry according to the Hungarian* and German Pharmacopoeia**. Means (n=3) and ± SDs are shown.

	Total tannin content*		Total flavonoid content**	
	Plant drug (%)	Extract (mg 100 mL^{-1})	Plant drug (%)	Extract (mg 100 mL^{-1})
S. officinalis Hungarian	8.15 ± 0.05[1]	66.3 ± 1.2[1]	7.82 ± 0.14	59.8 ± 0.7[3]
Transylvanian	7.88 ± 0.08[1]	62.4 ± 0.9[1]	7.85 ± 0.09	61.4 ± 0.8[3]
S. sclarea Hungarian	4.06 ± 0.06	28.5 ± 0.8	5.22 ± 0.08[2]	32.4 ± 0.5
Transylvanian	4.01 ± 0.04	27.9 ± 0.9	4.76 ± 0.12[2]	31.6 ± 1.1
S. pratensis Hungarian	4.16 ± 0.12	8.9 ± 0.4	5.26 ± 0.07	22.5 ± 0.6[4]
Transylvanian	4.08 ± 0.05	8.5 ± 0.3	5.30 ± 0.05	25.7 ± 0.7[4]
S. nemorosa Hungarian	3.89 ± 0.08	9.4 ± 0.5	2.91 ± 0.11	9.8 ± 0.5

Data for tannin [1] and flavonoid content [2, 3, 4] are significantly different at $p < 0.05$.

Table 2. Identified terpenes in leaves of *Salvia* species by GC-MS.

	S. officinalis	*S. sclarea*	*S. pratensis*	*S. nemorosa*
linalool	+	+		
linalyl acetate	+	+	+	
geraniol	+			+
borneol	+	+	+	+
bornyl acetate	+			+
isoborneol	+			
α-thujone	+	+	+	+
β- thujone	+		+	
α-pinene	+	+		+
β-pinene	+	+		+
camphene	+			
myrcene	+	+		
limonene	+	+		
eucalyptol	+	+		
α-terpineol	+	+		
β-caryophyllene	+		+	
caryophyllenol	+			
α-humulene	+			
γ-humulene		+		
α-farnesene	+			
γ-muurolene			+	

Table 3. Element concentrations (mg/kg) ± standard deviations of *Salvia* species.

Element	Hungarian				Transylvanian		
	S. officinalis	*S. sclarea*	*S. pratensis*	*S. nemorosa*	*S. officinalis*	*S. sclarea*	*S. pratensis*
Al*	120.0 ± 2.4	114.1 ± 1.9	684.0 ± 6.7	118.1 ± 1.4	487.6 ± 8.5	230.1 ± 7.0	309.4 ± 10.7
As	< 1.5	< 1.5	< 1.5	< 1.5	1.83 ± 1.14	2.03 ± 1.46	39.52 ± 0.62
B*	30.14 ± 0.40	6.35 ± 09	34.20 ± 0.23	38.36 ± 0.42	< 0.2	< 0.2	< 0.2
Ba*	17.63 ± 0.33	27.04 ± 1.1	16.56 ± 0.37	8.39 ± 0.07	30.38 ± 0.64	35.62 ± 1.57	15.66 ± 1.24
Ca*	23948 ± 74	13458 ± 225	18558 ± 268	8235 ± 108	11009 ± 180	8844 ± 298	11420 ± 290
Cd	< 0.1	< 0.1	0.161 ± 0.09	0.181 ± 0.054	< 0.1	< 0.1	< 0.1
Co	< 0.15	< 0.15	0.436 ± 0.342	< 0.15	< 0.15	< 0.15	< 0.15
Cr*	4.21 ± 0.35	4.51 ± 0.18	0.907 ± 0.023	1.44 ± 0.40	2.99 ± 0.47	2.55 ± 0.24	0.567 ± 0.270
Cu*	8.09 ± 0.18	119.2 ± 17	9.91 ± 0.09	14.24 ± 0.25	10.73 ± 0.48	9.17 ± 0.40	17.15 ± 0.54
Fe*	1055 ± 6	198.5 ± 2.9	683.5 ± 4.2	152.6 ± 2.4	246.1 ± 9.1	66.17 ± 3.78	327.6 ± 7.5
Hg	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	4.78 ± 0.50
K*	30663 ± 112	22465 ± 178	18321 ± 39	36812 ± 246	29260 ± 136	24267 ± 565	36774 ± 220
Li	< 0.15	< 0.15	1.74 ± 0.23	9.54 ± 0.09	< 0.15	< 0.15	< 0.15
Mg*	6967 ± 96	2205 ± 16	9473 ± 105	4638 ± 8	4428 ± 137	1841 ± 47	6281 ± 53
Mn*	53.94 ± 0.52	10.21 ± 0.12	39.93 ± 0.25	21.84 ± 0.34	33.05 ± 0.04	15.67 ± 0.91	27.49 ± 0.33
Mo	1.02 ± 0.41	< 0.1	< 0.1	< 0.1	< 0.1	1.18 ± 0.68	< 0.1
Na*	61.63 ± 0.84	168.9 ± 6.5	24.05 ± 1.07	134.6 ± 1.2	30.67 ± 1.96	45.96 ± 1091	65.3 ± 1.00
P*	2068 ± 24	1852 ± 45	2976 ± 22	3247 ± 77	3358 ± 2	3268 ± 52	4409 ± 22
Pb	< 1.5	< 1.5	2.20 ± 0.32	1.75 ± 0.44	< 1.5	2.47 ± 0.67	< 1.5
S*	2559 ± 11	17437 ± 11	2669 ± 7	3407 ± 27	3632 ± 147	2255 ± 23	3732 ± 53
Ti*	27.92 ± 1.51	9.45 ± 1.39	2.59 ± 0.25	1.64 ± 0.09	7.75 ± 0.12	1.02 ± 0.41	1.65 ± 0.32
Zn*	25.88 ± 0.25	17.34 ± 0.32	19.85 ± 0.19	29.39 ± 0.38	55.24 ± 2.96	31.99 ± 1.59	56.13 ± 0.75

* Element concentration of samples significantly differs at $p<0.05$.

Table 4. Element concentrations (μg $100mL^{-1}$) ± standard deviations of extracts of *Salvia* species. Dissolution rates (%) in parenthesis.

Element	Hungarian			Transylvanian			
	S.officinalis	S. sclarea	S. pratensis	S. nemorosa	S. officinalis	S. sclarea	S. pratensis
Al*	8.6 ± 0.1 (0.7)	19.4 ±0.3 (17.9)	86.6 ± 0.9 (12.7)	11.5 ± 0.6 (11.2)	57.0 ± 0.1 (11.5)	59.3 ±1.6 (25.1)	41.3 ± 0.13 (13.3)
B*	5.5 ± 0.4 (19.1)	0.21 ± 0.01(3.5)	6.0 ± 0.5 (17.6)	4.2 ± 0.2 (13.1)	< 0.03	< 0.03	< 0.2
Ba*	1.8 ± 0.1 (11.1)	3.5 ± 0.1 (13.4)	3.8 ± 0.1 (23.0)	2.1 ± 0.1 (30.1)	4.2 ± 0.1 (13.5)	6.4 ± 0.1 (17.4)	3.85 ± 0.1 (24.5)
Ca*	17530 ± 42 (76)	3113 ± 86 (24.3)	5213 ± 40 (28.1)	1240 ± 29 (18.0)	3331 ± 73 (29.8)	3748 ± 43 (41.2)	4879 ± 59 (42.7)
Cd	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.1
Co	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	< 0.15
Cr	< 0.03	< 0.03	0.35 ± 0.03 (38.6)	< 0.03	0.8 ± 0.1 (27.8)	0.6 ± 0.1 (24.5)	< 0.03
Cu*	< 0.03	5.6 ± 0.2 (5.0)	9.91 ± 0.09	3.7 ± 0.3 (30.9)	3.7 ± 0.2 (34.5)	4.2 ± 0.1 (45.0)	4.1 ± 0.21 (24.3)
Fe*	21.5 ± 0.1 (2.1)	6.5 ± 0.1 (14.7)	58.1 ± 0.5 (8.5)	4.4 ± 0.1 (3.4)	7.9 ± 0.1 (3.2)	11.2 ±0.3 (16.3)	13.7 ± 0.03 (4.2)
Hg	< 0.13	< 0.13	< 0.13	< 0.13	< 0.13	< 0.13	< 0.13
K*	26796± 99 (87.4)	5127±25 (24.0)	1791±284 (97.7)	5286± 59 (17.2)	25590±39 (86.2)	24400± 400 (97.8)	26372±361 (71.7)
Li	< 0.04	< 0.04	< 0.04	2.8 ± 0. 9 (35.9)	< 0.04	< 0.04	< 0.15
Mg*	877 ± 10 (13.1)	1037 ± 16 (49.5)	6093 ± 73 (64.3)	721 ± 12 (18.6)	2424 ± 34 (53.9)	1234 ± 19 (65.2)	3608 ± 32 (57.4)
Mn*	25.2 ± 0.2 (48.5)	0.79 ± 0.01(8.1)	10.7 ± 0.40 (26.8)	4.1 ± 0.1 (22.5)	7.2 ± 0.1 (21.33)	5.2 ±0.01 (31.9)	6.4 ± 0.1 (23.4)
Mo	0.18±0.01 (17.8)	< 0.35	< 0.35	< 0.1	< 0.35	0.38±0.15 (31.0)	< 0.1
Na*	48.0 ± 0.2 (80.9)	54.6 ±0.5 (33.9)	7.6 ± 0.1 (31.8)	102 ± 2 (90.4)	28.7 ± 2.0 (92.2)	29.8 ±0.6 (63.0)	42.3 ± 0.11 (64.7)
P*	989 ± 36 (49.7)	325 ± 5 (18.5)	1399 ± 12 (47.0)	355 ± 5 (13.1)	2466 ± 43 (72.3)	815 ± 7 (24.3)	1775 ± 12 (40.0)
Pb	< 0.3	< 0.3	0.35 ± 0.03 (16.1)	0.11 ± 0.01 (7.4)	< 0.3	< 0.3	< 1.5
S*	1125 ± 18 (45.6)	4537 ± 12 (27.4)	1962 ± 36 (73.5)	406 ± 13 (14.3)	2692 ± 43 (73.3)	1655 ± 70 (71.3)	2474 ± 53 (66.3)
Ti	< 0.03	< 0.03	0.72 ± 0.03 (27.7)	< 0.03	< 0.03	< 0.03	0.12 ± 0.01 (7.3)
Zn*	1.9 ± 0.6 (7.1)	4.9 ± 0.1 (30.0)	6.7 ± 0.06 (33.6)	3.8 ± 0.1 (15.7)	10.4 ± 0.4 (18.5)	10.9 ±0.1 (33.2)	16.7 ± 0.5 (29.7)

Element concentration of samples significantly differs at $p< 0.05$.

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Figures

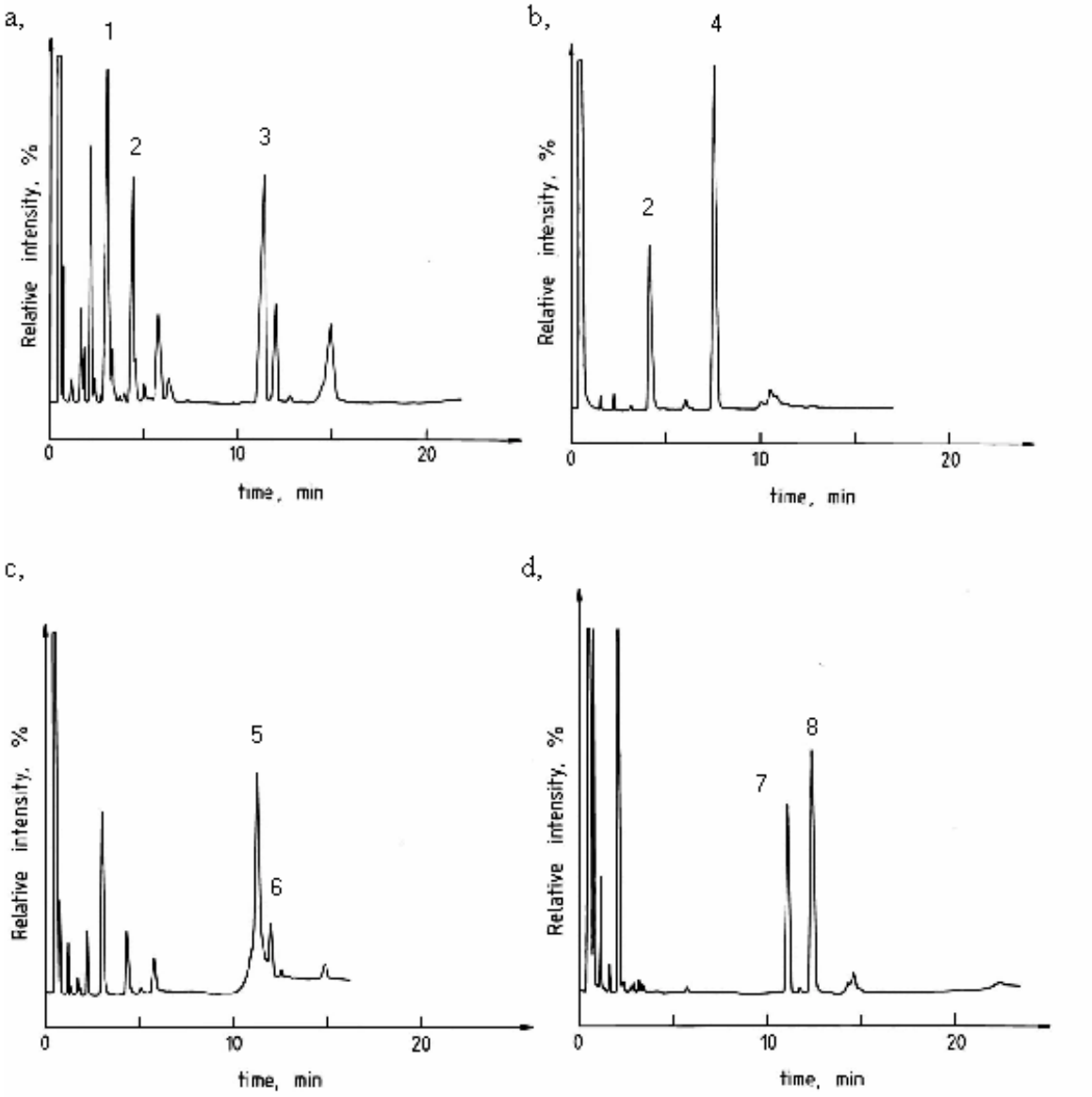

Fig. 1. Gas chromatograms of essential oils of *Salvia* species.
(1. α-thujone, 2. linalool, 3. α-humulene, 4. linalyl acetate, 5. β-caryophyllene, 6. γ-muurolene, 7.,8. unknown)
a, *Salvia officinalis* L.,
b, *Salvia sclarea* L.
c, *Salvia pratensis* L.
d, *Salvia nemorosa* L.