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TLC-Densitometry of Rosmarinic and Caffeic Acids in the Evaluation of Lamiaceae Species Growing in Central Europe

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Key Words

TLC, Desitometry, Rosmarinic acid, Caffeic acid, *Salvia* species, Lamiaceae

Summary

A TLC-densitometric method was used for the parallel quantification of rosmarinic acid (RA) and caffeic acid (CA) in crude extracts of *Salvia* species (Family Lamiaceae), obtained by ultrasonic extraction with 60% methanol. The densitometric measurement was performed in fluorescent mode as it has been published earlier. The applicability of the method has been investigated mainly from the viewpoint of the starting material. Questions are discussed like, what kind of factors should be taken into account, if the drugs are to be characterized, and how the RA and CA contents of samples vary in the plants are discussed. The drugs (plant material) show great differences due to the time of harvest of time, to the organ composition of drugs, to the extraction and storage conditions of the stock-solutions prepared from them. The importance of these parameters is illustrated on *Salvia* species native to Hungary.

1 Introduction

In the evaluation of medicinal plants or their drugs, it is not enough only to identify the presence of the active ingredients but the quantity of the compőounds present in the plants may be of importance. The content of the chemical ingredients in the plants, however, vary due to genetic and environmental circumstances. In these and many other cases TLC-densitometry may serve as a suitable technique. This method is fast and cheap and can be easily performed. Its precondition is that the evaluated components should be extracted in a rather pure form, and they should fulfil both the conditions of TLC separation and the requirements of densitometry. The compounds or their derivatives that are to be measured should have a suitable chromophore for light absorption (and light emission) of which should show linear concentration dependence. The method that was usedearlier for measuring rosmarinic acid (RA) and caffeic acid (CA) fulfils these requirements [1].

The Lamiaceae family comprises a lot of medicinal plants, many of which are in pharmacopoeias. Taxonomists have classified the family in different ways. According to one of the classifications the Lamiaceae consists of 2 subfamilies. Erdtman's system distinguishes Subfamily Lamioideae and Subfamily Nepetoideae [2]. Several chemical ingredients of the family show characteristic distribution in these subfamilies; for example iridoids and ecdysteroids are in the subfamily Lamioideae, whereas di- and

triterpenes, the ingredients of essential oils and phenolic compounds can be found in both subfamilies but they show different accumulation patterns. RA has been found almost exclusively in the species of the subfamily Nepetoideae, while CA seems to be ubiquitous in the whole family [3-6]. They are biologically active ingredients of the Lamiaceae species and also those of the drugs prepared from them [7-10]. It is easy to see that the determination of the content of these ingredients is of great importance in the course of the evaluation of plant materials and in qualifying the drugs. To attain these ends adequate methods should be applied. In many cases only a small amount of plant material is available and at the same time, because of various reasons, fast measurement is necessary. For such purposes the accuracy of TLC-densitometry may be acceptable [1,11-13]. We should keep in mind that plant materials themselves can vary significantly. This fact should also be taken into account in the evaluating processes (e.g. at the accuracy requirements of the measurement).

RA is a characteristic ingredient of the Lamiaceae species belonging to the Nepetoideae subfamily. CA can be found in the whole plant family but in much less concentration than RA. Both compounds contribute to the activities of the medicinal plants of the Lamiaceae family, predominantly because of their antioxidant capacities [9, 10]. In our former publications we also have given information about the subfamiliar distribution of RA and CA and have provided some quantitative data on their presence in many Lamiaceae species by using a TLC-densitometric assay [1,3,11-13]. The quantitative data on the drugs obtained by this method reflect both the differences due to the measuring processes and those due to the variation to plant sources. This time we concentrate on the latter question, on the influence of plant material on the quantitative data of RA and CA, predominantly in the evaluation of

Salvia species native to Hungary. On the one hand we are interested in how the RA and CA contents vary depending on some aspects of sample preparation and on the other hand how the organs and time of collection of the plants can influence the RA and CA content of drugs. Such type of information on the *Salvia* species native to Hungary has not been published on the RA and CA content.

2. Experimental

Plants were grown in the experimental field of the Research Institute of Botany and Ecology of the Hung. Acad. Sci. at Vácrátót (30 km north of Budapest) in various years. (Voucher samples were deposited in the herbarium collection of the institute.) After having separated the organs, the samples were dried at room temperature and ground by a household coffee mill. 0.4-0.5 g of the dried and powdered plant material was weighed and extracted with 7 ml of 60 % aqueous methanol for 10 min, using an ultrasonic extractor (Tesla, Czech). This procedure was repeated three times. The extracts obtained were combined and made up to 25 ml in a volumetric flask. Ready-made Kieselgel 60 Merck (Germany) plates were used (size 10x 20cm) for TLC. 20 µl samples from the stock-solution were spotted 10 mm from the buttom. Toluene – ethyl acetate – formic acid (5:4:1) solvent system served as developing agent in an unsaturated chamber [1, 14]. All solvents were of analytical grade. After developing (to approx. 80 mm length) the plates were dried for 20 min. at room temperature with a hair-drier and for an additional 20 min they were exposed to normal light in order to obtain better light emission [1]. In all cases RA and CA reference samples (Roth, Germany) were co-chromatographed with the plant samples. RA and CA show similar absorption in UV light and their emission spectra were also similar to each other. The

densitometric measurements were carried out at 325 nm. An IBM PC-controlled Shimadzu CS-9301 instrument was used in fluorescence mode, the scanning mode was linear, beam size: 10x 0.5 mm, wavelength 325, and the emission filter: I (cuts off the light below 350 nm). The plates were kept for 10-45 min in the instrument before scanning. The other details of the measurements and the method have been published [1].

Results and Discussion

TLC-densitometric measurements are suitable for the evaluation of the variation of RA and CA contents. The method is fast and economic regarding the chemicals and other necessary means. For the evaluation of plant sources it is acceptably reproducible. It should however, be emphasized that special care should be taken, if we want to extend the application of our method onto other plants. In such cases the main TLC-densitometric parameters should be controlled. This time we can confirm the applicability of our published method [1] at least for routine measurements in the *Salvia* species of the Lamiaceae family.

A typical densitogram obtained in the case of Salv*ia glutinosa* L. extract (Figure 1) illustrates the separation of the two compounds and the applicability of the method. Similar densitograms (similar separation) have been published on samples from *S.*

officinalis [1] and from *Hyssopus officinalis* [15] and were obtained in all cases of Lamiaceae taxa where these two compounds were present and were measured.

Figure 1.

The calibration plots of the 2 compounds are demonstrated by Figures 2 and 3. Both compounds provide a rather straight calibration plot in the marked intervals. Reproducibility (variation coefficients) of measurement for RA was 0.34 %, and 0.36 % for CA, if the spots were measured between 10-45 minutes (changes during 3 hours were not significant) [1].

Figure 2. and Figure 3

From a practical view point the length of the storage time of stock-solutions may be of importance. *Melissa officinalis* L., *Lavandula angustifolia* L., and *Salvia officinalis* L. solutions were stored in light at ambient temperature (Figure 4 and Figure 5) and in dark in a refrigerator at 4-5 ^{o}C, (Figure 6 and Figure 7) for 3 weeks so that we should see if there were any changes in the RA and CA content of extracts. The other storage conditions were the same in all samples. The means of RA and CA content of the samples were calculated on the bases of six parallel measurements. Figure 4 and 6 show the changes of RA and Figure 5 and 7 the changes of CA content respectively.

Figure 4-7

The stock-solutions could be stored for one day without significant changes. After longer the storage time, more changes could be observed. Decreases in content were more significant in the solutions kept in light (Figures 4 and 5) and were much less in those stored in the dark at low temperature (Figure 6 and 7). CA proved to be more

sensitive than RA. Remarkable differences could be observed, if the solutions of the three plants were compared. The least decrease was observed both in RA and CA content of *S. officinalis* extract and what is more, the CA content of this solution held in the dark at low temperature proved to be more at the end of the experiment than it had been at the start. This phenomenon may be in connection with the relatively slow decomposition of RA and CA as a possible intermediate product of the RA decomposition can accumulate in the *S. officinalis* solution.

The differences in the variation of solutions prepared from the three species belonging to three different Lamiaceae genera, seem to be remarkable. It is important however to mention that within one day no significant differences could be found in comparing the figures with those of the starting concentrations. This finding suggests that independently of the Lamiacea taxa in question, the extracts are similarly stable for some hours of storage. After a day, however, differences due to the origin of the solution can be seen. In our case *S. officinalis* provided the least, and *M. officinalis* the most sensitive solutions. Highly possibly the ingredients present, other than RA and CA in the various solutions, influence the variation (stability) of RA and CA content.

In Tables 1 - 4. *Salvia* species growing in Hungary are listed together with their RA and CA contents. Table 1 and Table 2 show the means of RA and CA content calculated by averaging the data obtained for organs independently of when the plants were collected. 'Drug' word refers to the whole above-ground material of the plant. (When Lamiaceae species are mentioned in pharmacopoeias in many cases the expression 'drug' refers to such material.) These tables show that in most cases the RA content is higher in the samples than the CA content. No clear-cut connections between organs

of various plant species can be established. Of the species *S. pratensis* and *S. glutinosa* proved to be the best source of RA and *S. austriaca* and *S. nutans* the poorest *S. officinalis*, the official drug of pharmacopoeias, is between the two mentioned groups. The RA content of the 'drugs' is highest in the leaves, followed by the generative organs (predominantly inflorescence).

Tables 3 and 4 show the means obtained for the monthly variation of drugs without separation of the organs. These tables reflect also the differences in the life cycles of plants. In the early stages of development the CA content is higher than later on during the vegetation period. No remarkable differences could be found among species in the variation of the CA content (Table 4). RA content varies also due to the differences in species. The highest data were provided by *S. glutinosa* (Table 3). They were high during the whole vegetation period. *S. pratensis* had high RA content at the beginning of vegetation but in accordance with its short vegetation period, in August only a small amount of RA and CA could be measured. More or less it may be a general conclusion that the most advantageous month for RA content is June.

Table 1-4

4. Conclusions

TLC-densitometric measurements are suitable for the evaluation of the variation of RA and CA contents. The method is fast and economic regarding the chemicals and other necessary means. For the evaluation of plant sources it is acceptably reproducible. It should however, be emphasized that special care should be taken, if we want to

extend the application of our method to other plants. In such cases the main TLC-densitometric parameters should be controlled. This time we can confirm the applicability of our published method [1] at least for routine measurements in the *Salvia* species of the Lamiaceae family. The data are of taxonomic importance having been obtained on the variation of Central European *Salvia* species. From the viewpoint of antioxidant capacity *S. glutinosa* and *S. pratensis* seem to be similar to S*. officinalis* as far as their RA contents are concerned. This finding may be of economic importance.

Acknowledgement

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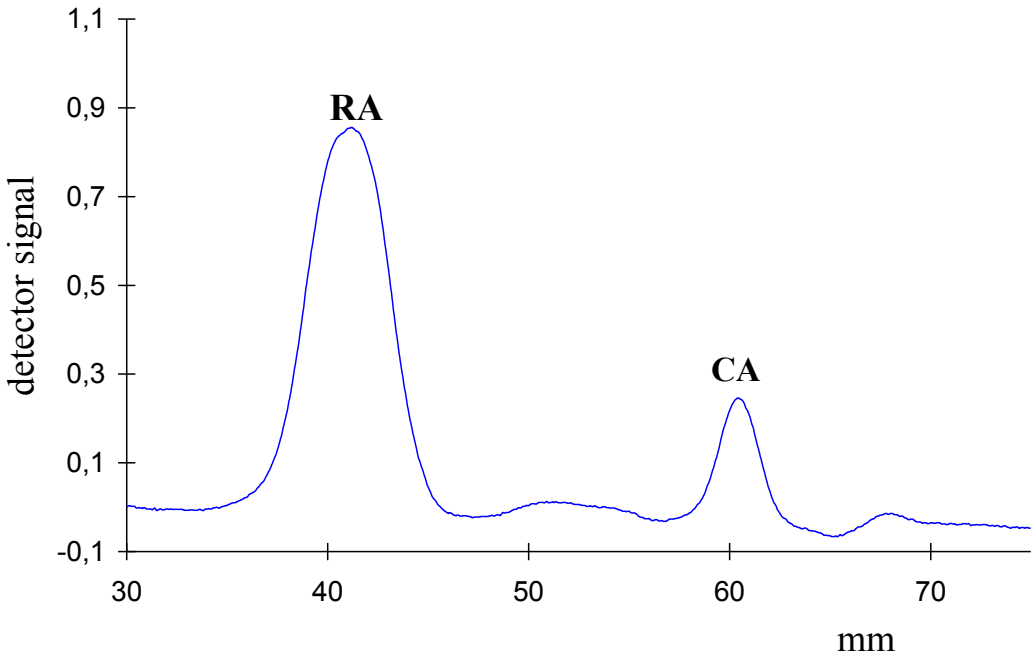

Figure 1

Densitogram of *Salvia glutinosa* extract RA: rosmarinic acid; CA; caffeic acid

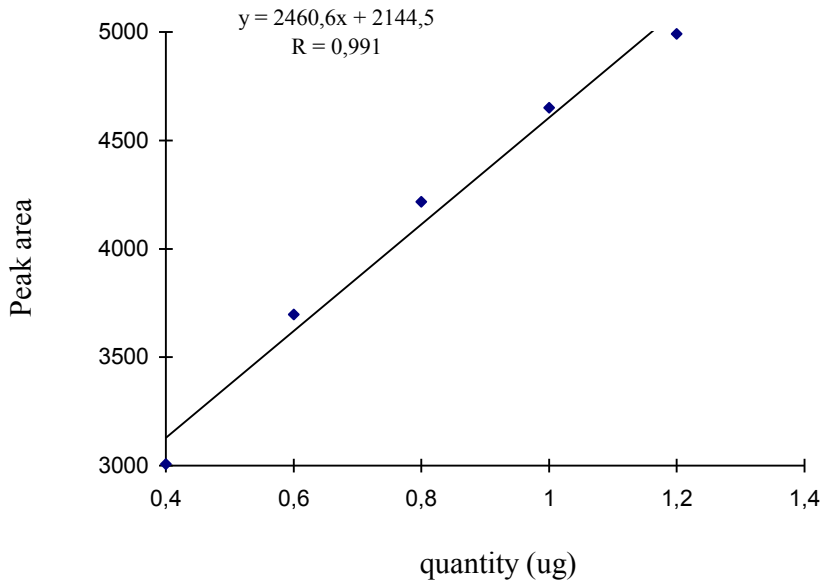

Figure 2

Calibration plot of caffeic acid (CA)

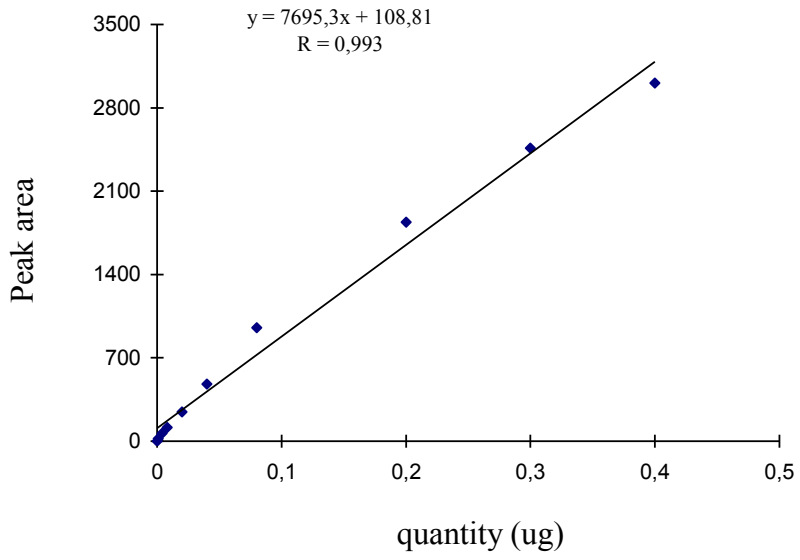

Figure 3

Calibration plot of rosmarinic acid (RA)

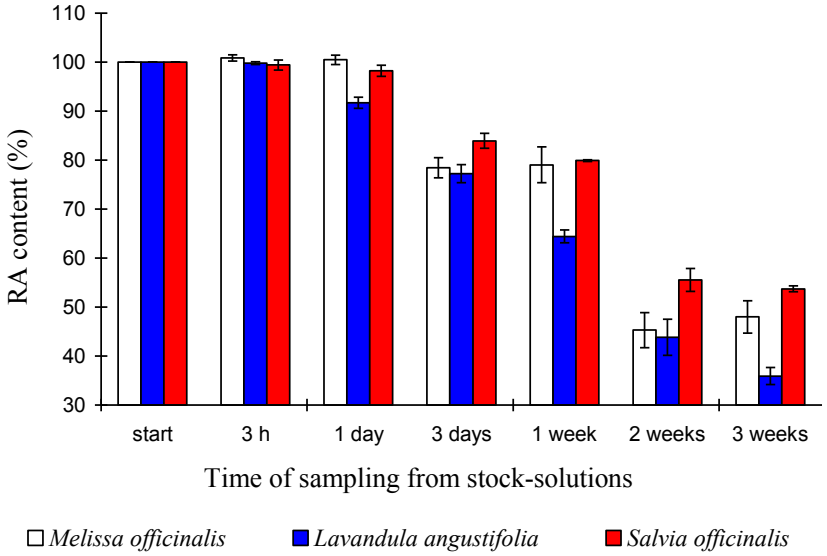

Figure 4

Changes of rosmarinic acid (RA) content of stock–solutions stored in light

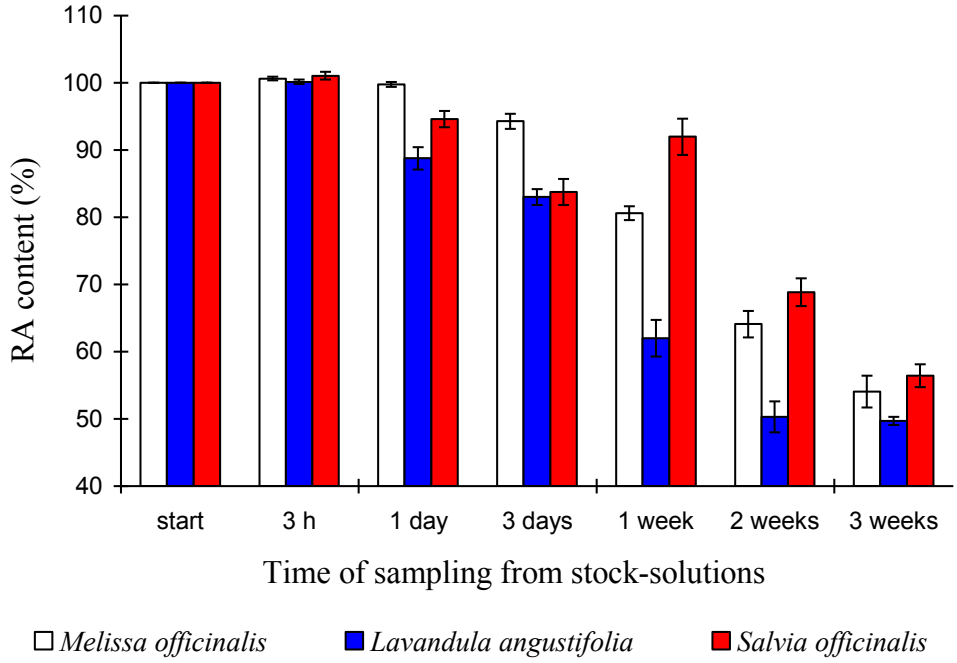

Figure 6

Changes of rosmarinic acid (RA) content of stock–solutions stored in dark

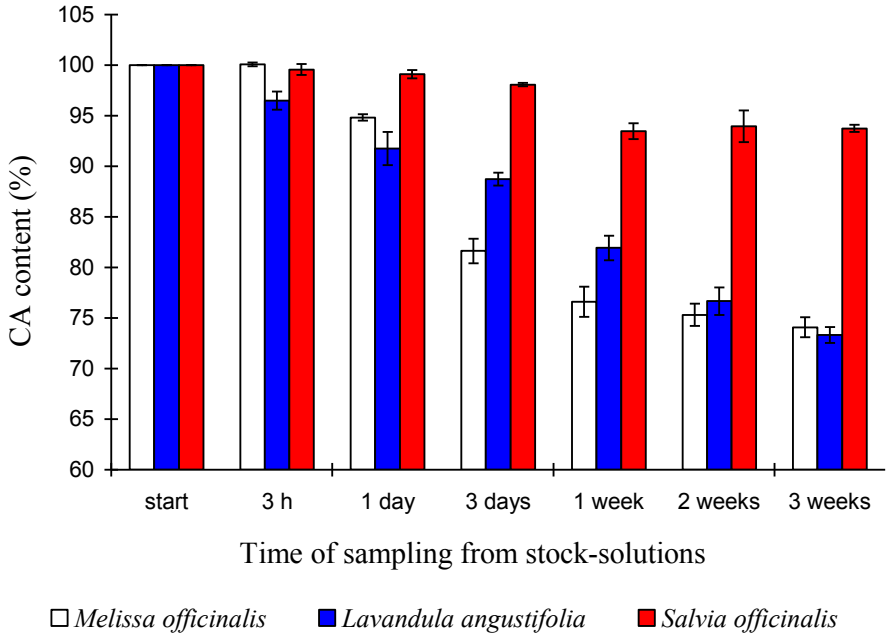

Figure 5

Changes of caffeic acid (CA) content of stock–solutions stored in light

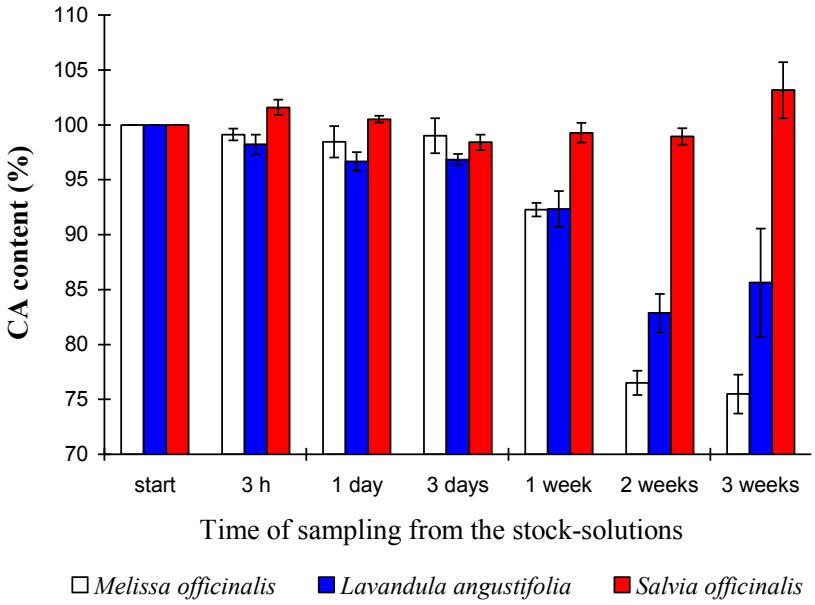

Figure 7

Changes of caffeic acid (CA) content of stock–solutions stored in dark

Table 1. Rosmarinic acid (RA) content (percent of dry weight) of organs of *Salvia* species

as an average for the vegetation period

Plant	Drug	Leaf	Stem	Generative parts
S. aethiopis L.	0.315	0.074	0.264	0.574
S. austriaca Jacq.	0.018	0.058	0.007	0.048
S. glutinosa L.	0.454	0.508	0.253	0.173
S. nemorosa L.	0.200	0.155	0.155	0.347
S. nutans L.	0.030	0.021	0.011	0.162
S. officinalis L.	0.312	0.178	0.300	0.319
S. pratensis L.	0.537	0.375	0.257	0.448
S. verticillata L.	0.388	0281	0229	0.344

Table 2. Caffeic acid content (percent of dry weight) of organs of *Salvia* species

as an average for the vegetation period

Plant	Drug	Leaf	Stem	Generative organs
S. aethiopis L.	0.021	0.026	0.014	0.057
S. austriaca Jacq.	0.027	0.034	0.006	0.010
S. glutinosa L.	0.056	0.060	0.270	0.023
S. nemorosa L.	0.200	0.015	0.010	0.033
S. nutans L.	0.004	0.005	0.002	0.009
S. officinalis L.	0.030	0.032	0.018	0.026
S. pratensis L.	0.050	0.057	0.012	0.024
S. verticillata L.	0.024	0.046	0.011	0.014

Table 3. Rosmarinic acid content of *Salvia* species (percent of dry weight)

Plant	April	May	June	July	August
S. aethiopis	0.357	0.022	0.092	0.180	0.004
S. austriaca	0.153	0.057	0.133	0.001	0.001
S. glutinosa	0.926	0.474	0.346	0.501	0.725
S. nemorosa	0.040	0.007	0.191	0.578	0.025
S. nutans	0.094	0.015	0.008	0.001	0.006
S. officinalis	0.520	0.159	0.148	0.199	0.034
S. pratensis	0.459	0.307	0.53	0.864	0.076
S. verticillata	0.536	0.237	0.868	0.755	0.347

Table 4. Caffeic acid content of *Salvia* species (percent of dry weight)

Plant	April	May	June	July	August
S. aethiopis	0.131	0.029	0.010	0.007	0.005
S. austriaca	0.119	0.070	0.014	0.002	0.001
S. glutinosa	0.221	0.090	0.018	0.020	0.008
S. nemorosa	0.045	0.005	0.009	0.013	0.001
S. nutans	0.001	0.012	0.007	0.005	0.001
S. officinalis	0.103	0.058	0.015	0.012	0.002
S. pratensis	0.173	0.074	0.031	0.057	0.005
S. verticillata	0.092	0.013	0.020	0.015	0.008