Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 101 (2007) 1748–1752
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Determination of carnosic acid and rosmarinic acid in sage by capillary electrophoresis

Selda Başkan, Nevin Öztekin, F. Bedia Erim *

Department of Chemistry, Technical University of Istanbul, Maslak, 34469 Istanbul, Turkey

Received 13 July 2005; received in revised form 9 January 2006; accepted 20 January 2006

Abstract

A simple and rapid capillary electrophoresis method was developed for the identification and quantitative determination of two antioxidative compounds – carnosic acid and rosmarinic acid – in the extracts of commercial Sage (*Salvia officinalis*) tea-bags. Capillary zone electrophoretic separation of carnosic and rosmarinic acids was performed using 40 mmol/l borate, at pH 9.6 as the running buffer. Weighed sage samples were extracted from tea-bags by sonification and the extracts were directly injected without any purification and pre-separation process. Coumarin was used as internal standard for quantitation and the limits of detection for carnosic acid and rosmarinic acid were obtained as 2.79 and 3.18 μg/ml, respectively using UV detection at 210 nm.

Keywords: Sage; *Salvia officinalis*; Carnosic acid; Rosmarinic acid; Capillary electrophoresis

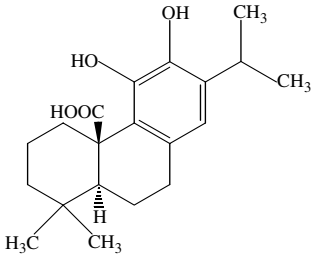
1. Introduction

Sage (*Salvia officinalis*) is a common herbal plant in the family Lamiaceae, cultivated and widely used in Turkey for its medicinal properties and for culinary purposes. As a herbal tea, sage is a popular beverage served widely in coffee houses and traditionally used to treat sore throats. Sage has been a subject of intensive study in order to identify its chemical compounds. The plant is a rich source of di- and triterpenoids, phenolic acids, and flavonoids (Lu & Foo, 2002; Ulubelen & Topçu, 1998). The main antioxidative effect of sage has been reported to relate to the presence of carnosic acid and rosmarinic acid (Cuvelier, Berset, & Richard, 1996) (Fig. 1). The level of both compounds in the dry leaves varies widely depending on genetic factors and environmental conditions. It is therefore important to develop a simple and reliable analysis method for the active components to determine the antioxidative power and the quality of the wild plant.

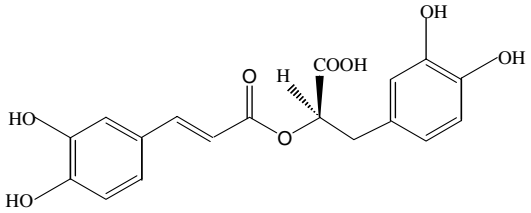
HPLC is a common method for the determination of antioxidant compounds in plant extracts. However, it generally necessitates the time and material consuming preliminary steps to prepare the sample before injection. Recently, capillary electrophoresis (CE) has been employed for the analysis of edible plants (Suntornsuk, 2002). The advantage of the capillary electrophoretic methods is the considerable diminutions in the sample preparation and analysis times, as well as in the reagent consumption. CE is particularly suitable in the analysis of complex natural matrices, owing to its higher resolving power. A few reports have been published on the capillary electrophoretic analysis of carnosic and rosmarinic acids in the rosemary extracts (Crego et al., 2004; Herrero et al., 2005; Ibañez et al., 2000; Sáenz-López, Fernández-Zurbano, & Tena, 2002), but only one of them (Bonoli, Pelillo, & Lercker, 2003) reported quantitative results, due to the lack of commercially available standards. Though a few works on the analysis of some active components in Salvia species using CE methods (Gu, Zang, Su, Chen, & Quyang, 2004; Hu, Jia, Zhang, Shi, & Wang, 1997; Zhao et al., 2000), to our knowledge, no research has been published on the analysis of carnosic and rosmarinic

* Corresponding author. Tel.: +90 21 22 85 31 51; fax: +90 21 22 85 63 86.
E-mail address: erim@itu.edu.tr (F.B. Erim).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.01.033



Carnosic acid



Rosmarinic acid

Fig. 1. Chemical structures of carnosic acid and rosmarinic acid.

acid contents in *Salvia* species using capillary electrophoretic methods.

In the present study, we describe the development of a simple and rapid capillary electrophoretic method for the simultaneous analysis of carnosic and rosmarinic acids for the routine control of their quantitative level. The developed method was applied to seven boxes of commercial tea-bags of sage obtained from local markets.

2. Materials and methods

2.1. Materials

Carnosic acid, rosmarinic acid, and methanol were purchased from Sigma (Steinheim, Germany). Sodium tetraborate and coumarin were from Merck (Darmstadt, Germany). Commercial sage tea samples were purchased from local markets. All solutions were prepared with deionized water purified in an Elgacan C114 (Elga, England) filtration system.

Carnosic acid and rosmarinic acid stock solutions were prepared by dissolving the acids in methanol. The injected solutions were prepared daily prior to use.

Five tea-bags from the same trademark were opened and mixed homogeneously in a container. The weighted sample was taken from this mixture. Before use, all solutions were filtered using a microfilter with a pore size of 0.45 μm.

2.2. Apparatus and operating conditions

Separations were performed with an Agilient capillary electrophoresis system equipped with a diode-array detector. The data processing was carried out with the Agilient ChemStation software. The wavelength was set at 210 nm. The separation was performed at 28 kV. Injections were made at 5×10^{-3} MPa for 5 s.

The fused silica capillaries used for separation experiments were 50 μm i.d. and were obtained from Polymicro Technologies (Phoenix, AZ, USA). The total length of the capillary was 53 cm and the length to the detector was 45 cm. The fused silica capillary was conditioned prior to use by rinsing with 1 mol/l NaOH for 30 min, water for 10 min, and buffer (at pH 9.6) with 10 min. The capillary was washed with 0.1 mol/l NaOH solution for 3 min and with buffer for 2 min between the runs.

3. Results and discussion

3.1. Separation of carnosic and rosmarinic acids

The structures of carnosic and rosmarinic acids shown in Fig. 1 suggest that they can be separated by capillary zone electrophoresis (CZE) mode at high pHs. Preliminary experiments showed that the standard samples of both acids can be separated in 9, 5 min with a good sensitivity when using a 40 mmol/l borate buffer at pH 9.6. The optimization of the separation of the two acids has been achieved by the choice of buffer concentration. To this end, the electropherograms for the two acids in the extract have been analyzed in the separation electrolyte in the interval of 10–60 mmol/l concentrations. In the concentrations below 40 mmol/l, the baseline separations of the small peaks seen at the left of rosmarinic acid in the electropherograms in Fig. 3 are not completely realized. In concentrations above 40 mmol/l, the separation time increases. Thus, 40 mmol/l has been chosen as the optimum separation electrolyte. During the experiments it was observed that the peak height of carnosic acid was decreasing during the storage of the standard solution and a new peak appeared before the carnosic acid peak. This peak was named as carnosol since there are many reported studies that carnosic acid is decomposed to carnosol by light and heat (Okamura, Fujimoto, Kuwabara, & Yagi, 1994; Thorsen & Hildebrandt, 2003). Fig. 2 shows the decomposition of the carnosic acid standard stored at 4 °C for one week. In one week 61% of carnosic acid was decomposed. However only 36% of carnosic acid was decomposed in the extract stored at 4 °C for one week. Thereby, the solutions used in the calibration process were prepared daily. The extraction, and the following filtration and injection process did not exceeded in 70 min. In that time interval, there was no detectable carnosol peak in the electropherogram.

Direct injections of the extracts after being filtered through a 0.45 μm filter were performed. A known amount of coumarin solution was added as internal standard to all injection samples. Fig. 3 shows the separation obtained for a sage extracts, and Figs. 4a–c show the on-line peak spectra of carnosic acid, rosmarinic acid, and carnosol respectively, which were obtained by the photodiode array detector under the chromatographic conditions described above. Peak identification was performed by spiking the samples with standard compounds and by UV spectral

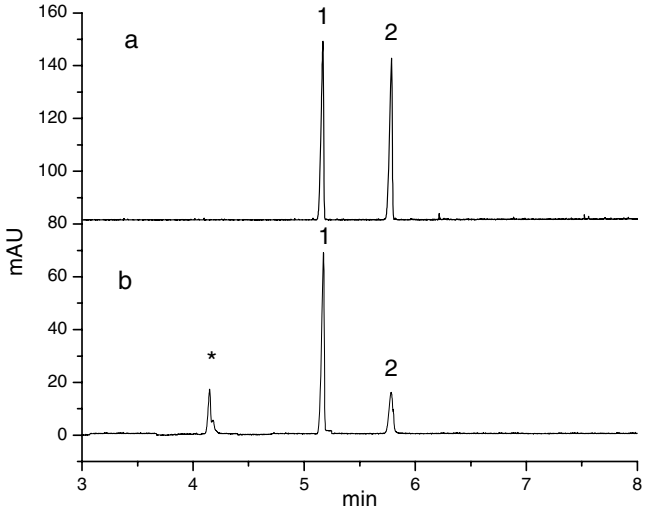


Fig. 2. Decomposition of carnosic acid in the standard solution. (a) Carnosic acid standard injected after preparation. (b) Injection of the same solution after stored at 4 °C for one week. Peaks: ∗, carnosol (decomposed from peak 2); 1, coumarin (internal standard); 2, carnosic acid.

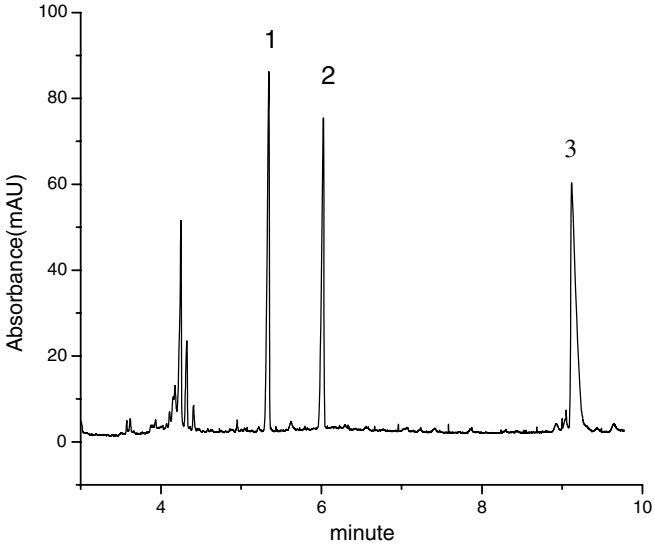


Fig. 3. Electropherogram of a sage extract. Buffer: 40 mM sodium borate at pH 9.6. Applied voltage 28 kV. Capillary 53 cm of total length, 45 cm of effective length with 50 μm i.d. Temperature 25 °C injection. 5×10^{-3} MPa for 5 s. Detection at 210 nm. Peaks: 1, coumarin (internal standard); 2, carnosic acid; 3, rosmarinic acid.

analysis. The purity of each peak in the tea samples was better than 99.8% as calculated from the peak purity spectrum.

3.2. Selection of solvent for the extraction of carnosic and rosmarinic acids

Water, methanol, acetonitrile, and acetone were used as the extraction solvent for carnosic and rosmarinic acids from sage samples. One gram of sage sample was dissolved

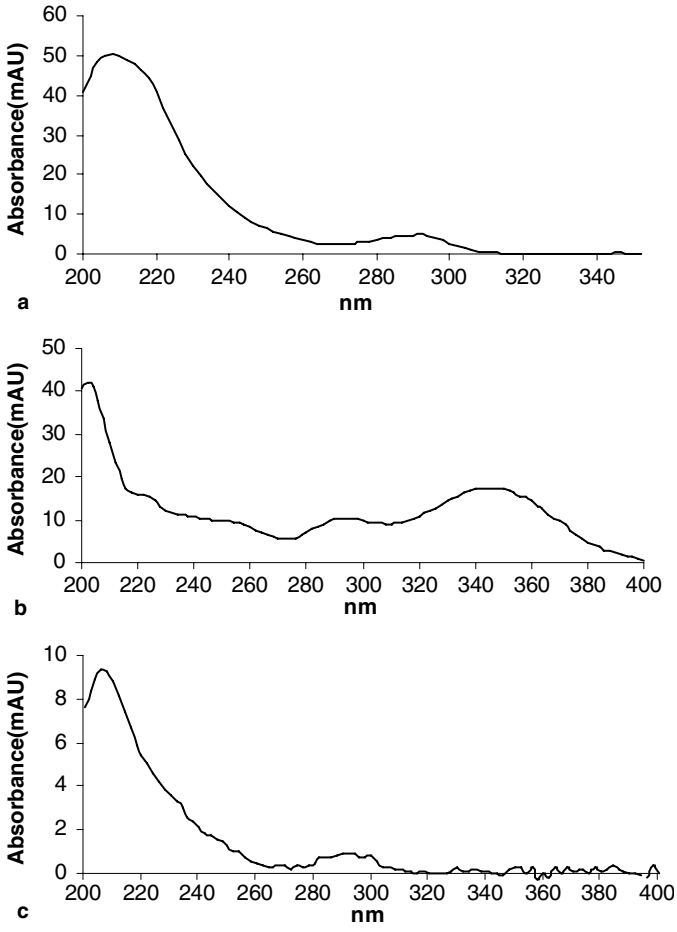


Fig. 4. (a) Spectrum of rosmarinic acid; (b) spectrum of carnosic acid; (c) spectrum of carnosol.

in 10 ml of each solvent and sonicated for 1 h. After filtration, the solutions were injected to the capillary. The peak areas were compared for each solvent. It was found that there was little difference between methanol, acetonitrile, and acetone for the solubility of carnosic acid. However carnosic acid was not detected in the water extraction solution. Rosmarinic acid was detected in all the solvents, but the solubility in methanol solvent was considerably more than in the other solvents. Fig. 5 shows the peak areas of carnosic and rosmarinic acids when four extraction solutions in different solvents were injected to the capillary. Based on Fig. 5, methanol was used to prepare the sage extracts.

3.3. Selection of extraction time

Figs. 6a and b show the effect of extraction time on the content of carnosic and rosmarinic acids. The sage sample was sonicated in the methanol for different sonication times. Each solution was injected to the capillary and relative peak areas were compared. After 1 h, the peak areas for both acids did not change, so that 1-h of sonication was applied for all the samples.

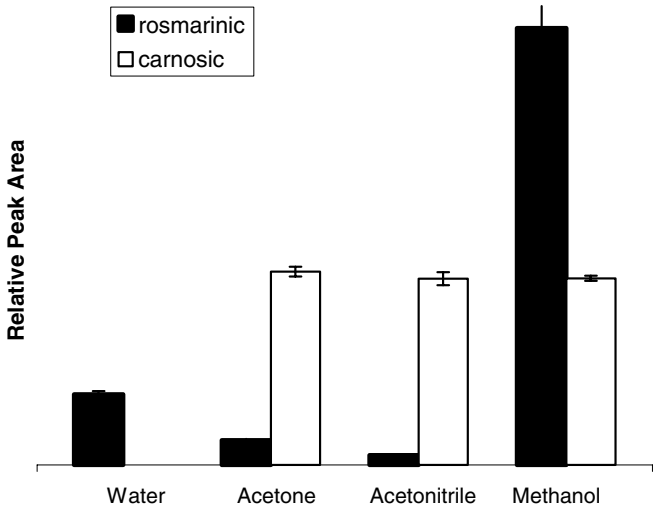


Fig. 5. Comparison of solvents for the extraction of carnosic and rosmarinic acids.

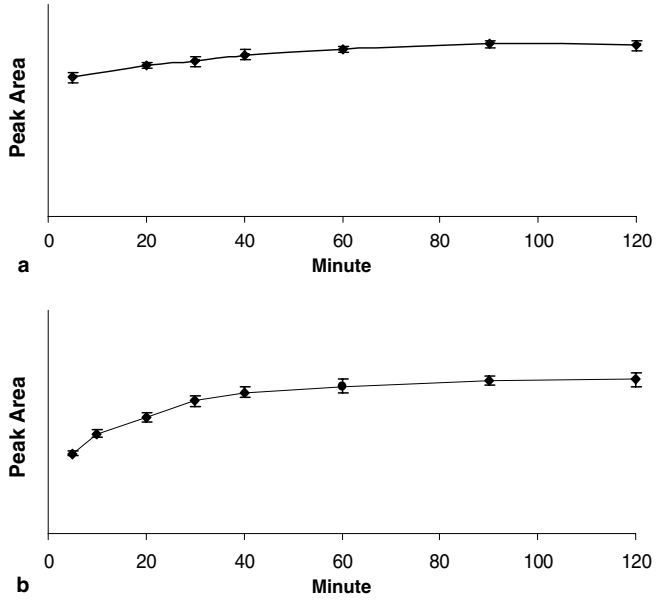


Fig. 6. The effect of extraction time on the content of: (a) carnosic acid; and (b) rosmarinic acid.

3.4. Comparison of sonication with refluxion

One gram of sage was refluxed by methanol for 1 h. The extracted amount of both acids was compared with the sonicated samples. As seen from Fig. 7, while there is no important difference in the amount of rosmarinic acid for two methods, the amount of carnosic acid was found 13% lower after reflux process than in the sonicated extracts.

In the all preliminary experiments to determine the extraction conditions, the same *Salvia* bag as the trade mark has been used. In every experiment of these three series, internal standards have been used and relative peak areas have been compared. The variance values are the

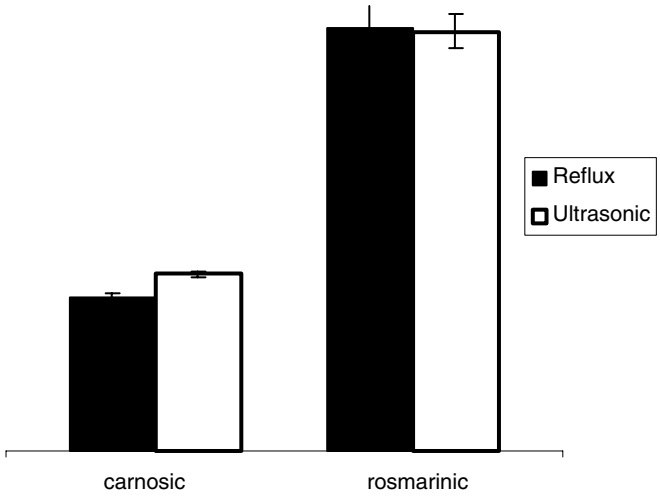


Fig. 7. Comparison of sonification with reflux as the extraction method.

low values between 1.8% and 3.5% for carnosic acid, and 3.0% and 5.0% for rosmarinic acid. No systematic difference between the series is detected.

4. Validation of the method

4.1. Linearity range and calibration curve

The linearity for carnosic and rosmarinic acids was checked using nine levels of concentrations. The linearity ranges for carnosic acid and rosmarinic acid were determined to be 20–700 and 20–800 μg/ml, respectively. The regression equations for carnosic and rosmarinic acids were $y = 0.43650186x - 0.0038267$ ($r = 0.99866$) and $y = 0.5610757x + 0.0335423$ ($r = 0.99797$), respectively.

4.2. Limits of detection

The limit of detection (LOD) was obtained as the concentrations of the acids that caused a peak with a height three times the baseline noise level. LOD was calculated as 2.79 and 3.18 μg/ml for carnosic and rosmarinic acids, respectively.

4.3. Precision

The precision of method was calculated as the coefficient of variation (CV) of migration times and peak areas for seven successive injections of standard samples. Repeatability of migration times for carnosic and rosmarinic acids were 0.09% and 1.03%, respectively, and the relative corrected peak areas (A/A_i) were 2.99% and 1.86%, respectively.

4.4. Recoveries

To check the percent recovery, a standard solution of each acid at different concentrations was added together with the internal standard solution to the extracted sage

Table 1
Recoveries of carnosic acid and rosmarinic acid

Added amount (μg/ml)	Carnosic acid (%) ($n = 2$)	Rosmarinic acid (%) ($n = 2$)
50	98.5 ± 2.5	93.6 ± 3.3
100	99.2 ± 3.0	95.1 ± 4.5
150	103 ± 1.4	109 ± 4.0

Table 2
Amounts of carnosic acids and rosmarinic acids (mg/g) in the seven commercial sage tea samples

Sample	Carnosic acid (mg/g)	Rosmarinic acid (mg/g)
1	5.42 ± 0.06	2.82 ± 0.16
2	7.16 ± 0.16	3.18 ± 0.22
3	6.31 ± 0.05	2.61 ± 0.24
4	2.99 ± 0.01	2.96 ± 0.14
5	3.45 ± 0.04	2.50 ± 0.01
6	6.81 ± 0.23	3.89 ± 0.36
7	4.67 ± 0.15	4.01 ± 0.4

solutions. Each concentration level repeated two times. Concentrations and recoveries are given in Table 1.

4.5. Quantitative measurements for sage samples

Table 2 reports the amounts of carnosic and rosmarinic acids in sage teas. Each value was the mean of three extractions, performed in different days. The content of carnosic acid ranged from 2.99 to 7.16 mg/g, and rosmarinic acid from 2.50 to 4.01 mg/g. The samples of numbers 1 and 5 were extracted three times in the same day. Carnosic and rosmarinic acid contents and standard deviations of the samples numbered 1 and 5 extracted in same day do not differ from extracts done over different days. Carnosic acid content is 5.42 ± 0.08 and 3.41 ± 0.03 and rosmarinic acid 2.87 ± 0.23 and 2.51 ± 0.01, for samples 1 and 5, respectively. The somewhat higher variance in certain tea samples is probably due to the fact that the particle sizes are different in different bags.

5. Conclusion

CE is a simple and rapid technique for the identification and determination of antioxidant compounds, carnosic and rosmarinic acids, in sage. The method does not require a pre-separation process. The main advantages of fused silica capillaries compared to packed columns are that plant extracts are directly injected without any purification step, easily washed between runs and free of irreversible contamination of the matrix. The method promises to be applicable to the quality control of the plant.

Acknowledgements

We thank the TUBITAK (Grant TBA6-2312, 103T053) and research foundation of Istanbul Technical University for financial support.

References

Bonoli, M., Pelillo, M., & Lercker, G. (2003). Fast separation and determination of carnosic and rosmarinic acid in different rosemary (*Rosmarinus officinalis*) extracts by capillary electrophoresis with Ultra Violet-Diode Array detection. *Chromatographia, 57*, 505–512.

Crego, A., Ibáñez, E., Garcia, E., de Pablos, R. R., Señoráns, F. J., Reglero, G., et al. (2004). Capillary electrophoresis separation of rosemary antioxidants from subcritical water extracts. *European Food Research and Technology, 219*(5), 549–555.

Cuvelier, M. E., Berset, H., & Richard, H. (1996). Antioxidative activity of phenolic compounds of pilot-plant and commercial extracts of sage and rosemary. *Journal of American Oil Chemical Society, 73*, 645–652.

Gu, M., Zang, S., Su, Z., Chen, Y., & Quyang, F. (2004). Fingerprinting of *Salvia miltiorrhiza* Bunge by non-aqueous capillary electrophoresis compared with high-speed counter-current chromatography. *Journal of Chromatography A, 1057*, 133–140.

Herrero, M., Arráez-Román, D., Segura, A., Kenndler, E., Gius, B., Raggi, M. A., et al. (2005). Pressurized liquid extraction-capillary electrophoresis-mass spectrometry for the analysis of polar antioxidant in rosemary extracts. *Journal of Chromatography A, 1084*, 54–62.

Hu, Z. D., Jia, L., Zhang, Z. P., Shi, Y. P., & Wang, K. T. (1997). Studies of crude ethanol extract of roots of *Salvia przewalskii* maxim by micellar electrokinetic capillary chromatography. *Journal of Liquid Chromatography and Related Technologies, 20*, 1211–1220.

Ibañez, E., Cifuentes, A., Crego, A. L., Señoráns, F. J., Cavero, S., & Reglero, G. J. (2000). Combined use of supercritical fluid extraction, micellar electrokinetic chromatography, and reverse phase high performance liquid chromatography for the analysis of antioxidants from rosemary (*Rosmarinus officinalis L.*). *Journal of Agricultural and Food Chemistry, 48*, 4060–4065.

Lu, Y., & Foo, L. Y. (2002). Polyphenolics of Salvia. *Phytochemistry, 55*, 263–267.

Okamura, N., Fujimoto, Y., Kuwabara, S., & Yagi, A. (1994). High-performance liquid chromatographic determination of carnosic acid and carnosol in *Rosmarinus officinalis* and *Salvia officinalis*. *Journal of Chromatography A, 679*, 381–386.

Sáenz-López, R., Fernández-Zurbano, P., & Tena, M. T. (2002). Capillary electrophoretic separation of phenolic diterpenes from rosemary. *Journal of Chromatography A, 953*, 251–256.

Suntornsuk, L. (2002). Capillary electrophoresis of phytochemical substances. *Journal of Pharmaceutical and Biomedical Analysis, 27*, 679–698.

Thorsen, M. A., & Hildebrandt, K. S. (2003). Quantitative determination of phenolic diterpenes in rosemary extracts – aspects of accurate quantification. *Journal of Chromatography A, 995*, 119–125.

Ulubelen, A., & Topçu, G. (1998). Chemical and biological investigations of Salvia species growing in Turkey. *Studies in Natural Products Chemistry, 20*, 659–718.

Zhao, Y. K., Cao, Q. E., Chen, X. G., Hu, Z. D., Fu, B., & Chang, J. M. (2000). Electrophoretic behaviour and quantitative analysis of active components 6,7-dehydroroyleanone an taxoquinone extracted from Salvia deserta Schang. *Chemia Analityczna, 45*, 237–248.