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RESEARCH ARTICLE

Investigations on Rosmarinic, Chlorogenic and Caffeic Acid Contents of *Salvia virgata, Salvia verticillata* ssp. *amasiaca* and Five Commercial *Salvia* Tea Bag Samples Using HPLC-DAD Method

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Investigations on Rosmarinic, Chlorogenic and Caffeic Acid Contents of Salvia virgata, Salvia verticillata ssp. amasiaca and Five Commercial Salvia Tea Bag Samples Using HPLC-DAD Method

SUMMARY

Salvia virgata and Salvia verticillata ssp. amasiaca together with five commercial tea bag samples were analyzed for their rosmarinic, chlorogenic, and caffeic acid content. HPLC-DAD analysis allowed the identification and quantification of phenolic acids in the methanol extracts. Results of the study revealed that the studied Salvia species are particularly rich in rosmarinic acid and commercial tea bag samples belonging to different firms have the similar phenolic acid profile with significant differences in terms of rosmarinic acid content.

Key Words: *Salvia, Sage, Rosmarinic acid, HPLC-DAD, Tea bag*

Salvia virgata, Salvia verticillata ssp. amasiaca ve Beş Ticari Adaçayı Poşet Çay Örneğinin Rozmarinik, Klorojenik ve Kafeik Asit İçerikleri Üzerinde YPSK-DAD Yöntemi ile İncelemeler

ÖZET

Salvia virgata, Salvia verticillata ssp. amasiaca ve beş ticari adaçayı poşet çay örneği rozmarinik, klorojenik ve kafeik asit içerikleri bakımından incelenmiştir. Metanollü ekstrelerdeki fenolik asitlerin teşhis ve miktar tayini YPSK-DAD ile gerçekleştirilmiştir. Çalışmanın sonuçları, incelenen Salvia türlerinin özellikle rozmarinik asit açısından zengin olduğunu, farklı firmalara ait ticari poşet çay numunelerinin ise benzer fenolik asit profiline sahip oldukları halde, rozmarinik asit içerikleri bakımından önemli farklılıklar gösterdiğini ortaya koymaktadır.

Anahtar kelimeler: *Salvia, Adaçayı, Rozmarinik asit, YPSK-DAD, Poşet çay*

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INTRODUCTION

Rosmarinic acid is a phenolic compound (caffeic acid ester) found in a variety of plants of the Boraginaceae and the subfamily Nepetoideae of the Lamiaceae. Some fern and hornwort species also contain rosmarinic acid as well as the species of some other higher plant families (1). *Salvia* species are the well-known rosmarinic acid sources and wide spread in Turkey, while they are important industrial plants used both for food and pharmaceutical purposes (2,3). Plant extracts, generally used for their flavoring characteristics, often have strong radical scavenging activity thus making them extremely effective antioxidants. This antioxidant activity is most often due to phenolic acids and the antioxidant potential of *Salvia* species is mostly due to rosmarinic acid which is usually the main constituent of the species (4-6). For this reason, qualitative and quantitative determination of rosmarinic acid in *Salvia* preparations is very important.

Tea bags containing plant materials in single or combined formulations are very popular and they are widely sold in all markets. The quality control studies and the identification assays for these tea samples are quite important and inadequate. For this purpose, rosmarinic acid levels of commercial sage tea bag samples as well as caffeic and chlorogenic acid levels were determined using a previously validated High Performance Liquid Chromatography-Diode Array Dedector (HPLC-DAD) method (7). Therefore, in the current work, a quantification study has been set out in order to compare the phenolic acid profiles of two *Salvia* species (*Salvia virgata* and *Salvia verticillata* ssp. *amasiaca*) and five commercial *Salvia* tea bag samples and to evaluate their rosmarinic acid contents.

MATERIALS AND METHODS

Plant Materials

Salvia virgata Jacq. (AEF 26746) *and Salvia verticillata* L. ssp. *amasiaca* (Freyn & Bornm.) Bornm. (AEF 26747) were collected from Ankara during their flowering period and voucher specimens were deposited in the Herbarium of the Ankara University Faculty of Pharmacy. Five boxes of commercial *Salvia* tea bag samples (twenty samples in each box of each brand name) were purchased from local supermarket in Ankara.

Chemicals and Standards

Chromatographic grade double-distilled water, HPLC-grade methanol, acetonitrile, and analytical grade trifluoroacetic acid were used for HPLC analysis. Phenolic acids used as standards were purchased from Sigma (Germany): rosmarinic acid (536954), chlorogenic acid (C3878), caffeic acid (C0625). All other chemicals were analytical grade and obtained from either Sigma or Merck.

Extraction

200 mg of the dried and grounded flowers, leaves and roots were extracted with methanol, using a magnetic stirrer, for 1 h (50°C, 250 rpm). Each extract was then filtered, completed to 10.0 mL in a volumetric flask with methanol, passed through a 0.45 µm filter, and applied to the HPLC system. The same extraction procedure was applied to the tea bag samples purchased.

Identification and Quantification of Phenolic Acids using HPLC-DAD

HPLC Conditions

The qualitative and quantitative analyses of the phenolic acids in the extracts were performed according to the following procedure (7). The analysis was performed with a LC system consisting of a HP Agilent 1260 series quaternary pump, degasser and photodiode array detector. ACE column (5 µ, 250 mm × 4.6 mm) was used at 30°C. The system was controlled and data analysis was performed with Agilent ChemStation software. All calculations concerning the quantitative analysis were performed with external standardization by measurement of the peak areas. Gradient elution was applied with a flow rate of 0.8 mL/min. The mobile phase was a mixture of trifluoroacetic acid 0.1% in water (solution A), trifluoroacetic acid 0.1% in methanol (solution B), and trifluoroacetic acid 0.1% in acetonitrile (solution C). The composition of the gradient was (A:B:C), 80:12:8 at 0 min, 75:15:10 at8 min, 70:18:12 at 16 min,65:20:15 at 24 min, 50:35:15 at 32 min, 25:60:15 at 40 min and 80:12:8 at 45 min. Duration between runs was 2 min. From each solution and sample, 10 µL was injected into the column and the chromatograms were recorded from 200 to 400 nm. Quantification was performed by measuring at 330 nm for all investigated phenolic acids using a photo-diode array detector. The chromatographic run time was 45 min.

Calibration

Five concentrations of rosmarinic, chlorogenic and caffeic acids were prepared in methanol ranging between 4.3-215 µg/mL, 5-250 µg/mL and 5.2-260 µg/mL, respectively. Triplicate 10 µL injections were made for each standard solution to see the reproducibility of the detector response at each concentration level. The peak areas obtained from injections were plotted against the concentrations to establish the calibration graph.

Limits of Detection and Quantification

Limit of detection (LOD) was established at a signal to noise ratio (S/N) of 3. Limit of quantification (LOQ) was established at a signal to noise ratio (S/N) of 10.

LOD and LOQ were experimentally verified by nine injections of phenolic acids in LOQ concentrations.

Precision

The precision of the method (within–day variations of replicate determinations) was checked by injecting nine times of phenolic acids at the LOQ level. The area values were recorded and RSD% was calculated.

Recovery

The spike recovery was carried out by the standard addition method. For determination of the recovery from the methanol extracts, three concentrations of the standard compounds were added prior to extraction. In each additional level, three determinations were carried out and mean value of recovery percentage was calculated.

RESULTS AND DISCUSSION

All the tea bag samples showed a similar phenolic profile, presenting differences only in the quantities found of each compound (Figure 1). Rosmarinic acid was the most abundant compound, being higher in *S. verticillata* ssp. *amasiaca*, especially in the leaves. HPLC chromatograms of the flower, leaf and root extracts of *S. verticillata* ssp. *amasiaca* and overlaid UV spectra of rosmarinic acid in different parts of the plant were presented in Figure 2. HPLC chromatograms belonging to the flower, leaf and root extracts of *S. virgata* and overlaid UV spectra of rosmarinic acid in different parts of the plant were shown in Figure 3.

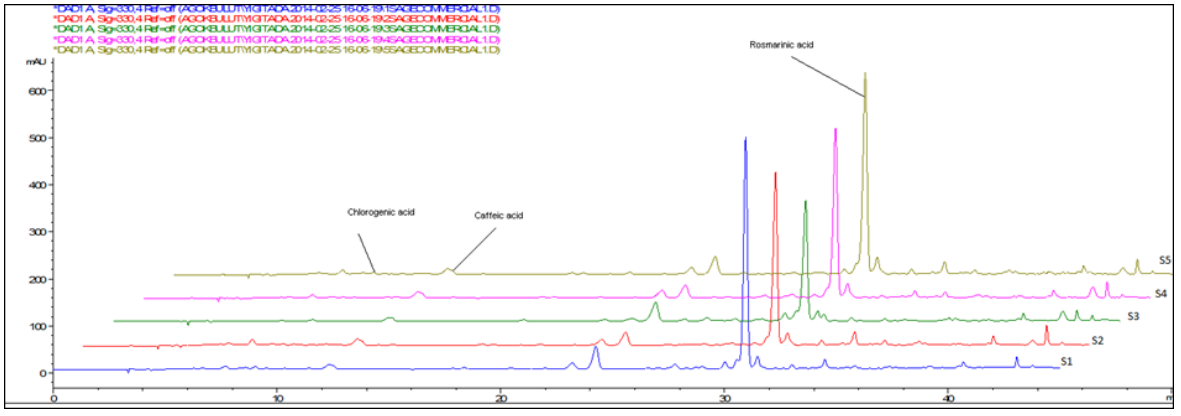

Figure 1. HPLC chromatograms of the studied tea bag samples.

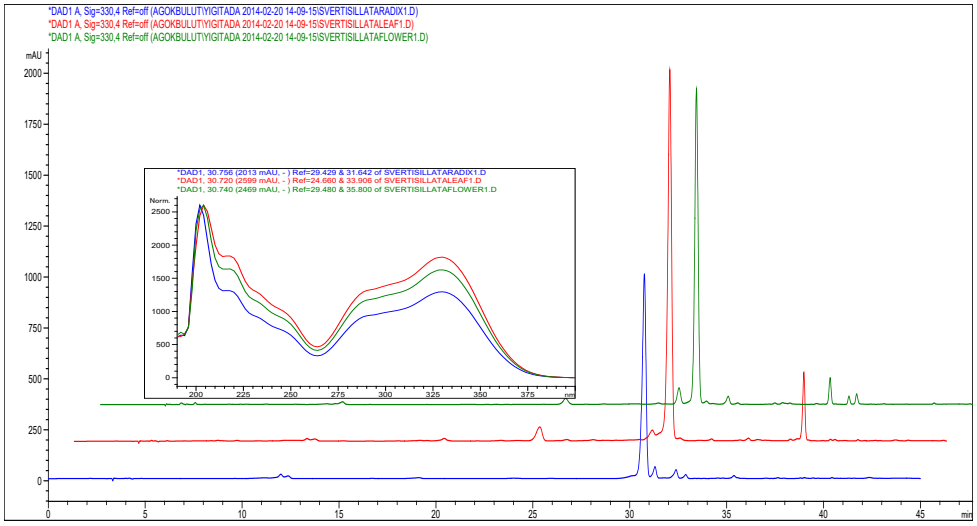

Figure 2. HPLC chromatograms of the flower, leaf and root extracts of *Salvia verticillata* ssp. *amasiaca* and overlaid UV spectra of rosmarinic acid in different parts of the plant.

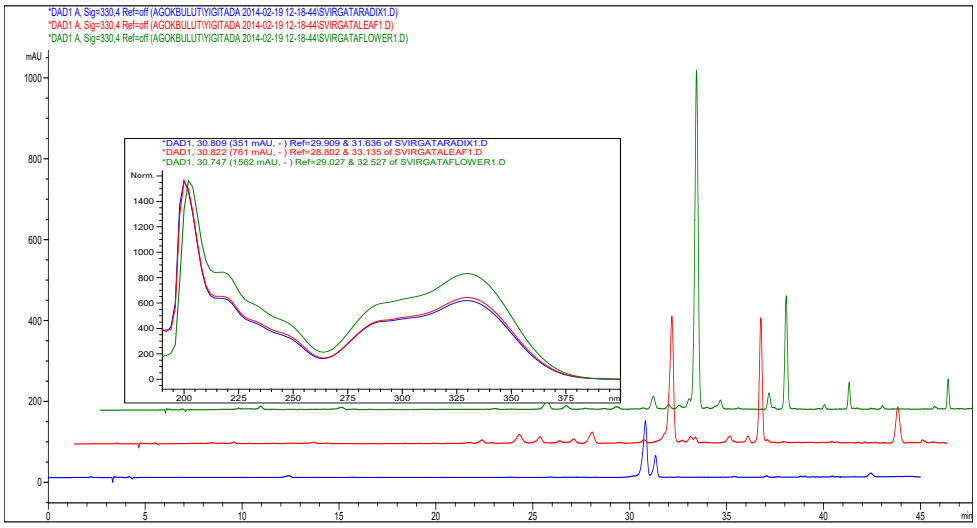

Figure 3. HPLC chromatograms of the flower, leaf and root extracts of *Salvia virgata* and overlaid UV spectra of rosmarinic acid in different parts of the plant.

The phenolic acid composition of the samples was compared in order to find out the possible variations between *Salvia* species and commercial samples (tea bags). According to the obtained results, the commercial tea bag samples of sage contained lower amount of rosmarinic acid considering the two *Salvia* species (Table 1). Not only the leaves, but also the flower and root extracts of *S. verticillata* ssp. *amasiaca* had really significant amount of rosmarinic acid among all the samples of *Salvia* and commercial samples investigated ranging between 2.466 ± 0.042% and 4.538 ± 0.074% (Table 2).

Analysis of the tea bag samples revealed that S1 and S5 had nearly the same amount of rosmarinic acid as 1.033 ± 0.011% and 1.002 ± 0.003%, respectively. For S1 and S5, although the brand names of the tea bag samples were different, the production units were the same, when the cover of tea bags examined carefully. It was clear that same plants were packed in the same production unit with different commercial names. S3 contained the lowest amount of rosmarinic acid among the five samples (0.634 ± 0.012%). The amount of rosmarinic acid in S2 and S4 samples were found to be quite close to each other as 0.853 ± 0.003% and 0.841 ± 0.003%, respectively.

Table 1. Phenolic acid content in the tea bag samples.

Sample	Rosmarinic acid %	Chlorogenic acid %	Caffeic acid %
S1	1.033 ± 0.011	0.014 ± 0.002	0.005 ± 0.001
S2	0.853 ± 0.003	0.009 ± 0.001	0.006 ± 0.001
S3	0.634 ± 0.012	0.012 ± 0.001	0.004 ± 0.001
S4	0.841 ± 0.003	0.007 ± 0.001	0.006 ± 0.001
S5	1.002 ± 0.003	0.014 ± 0.001	0.006 ± 0.001

Results were expressed as mean ± SD (n=3)

Table 2. Phenolic acid content in *Salvia virgata* and *Salvia verticillata* ssp. *amasiaca*.

Sample	Rosmarinic acid %	Chlorogenic acid %	Caffeic acid %
***S. virgata* flower**	1.780 ± 0.004	0.021 ± 0.001	0.005 ± 0.001
***S. virgata* leaf**	0.814 ± 0.011	0.012 ± 0.001	0.001 ± 0.001
***S. virgata* root**	0.369 ± 0.001	-	0.005 ± 0.001
***S. verticillata* ssp. flower**	3.332 ± 0.008	0.007 ± 0.001	0.016 ± 0.001
***S. verticillata* ssp. leaf**	4.538 ± 0.074	0.012 ± 0.001	0.013 ± 0.001
***S. verticillata* ssp. root**	2.466 ± 0.042	-	0.015 ± 0.001

Results were expressed as mean ± SD (n=3)

In this study, all the valuable data were obtained by a validated HPLC-DAD method and the detailed validation parameters were given in our previous study (7). Only a few studies have been reported so far for the determination of rosmarinic acid on several *Salvia* species (2, 3, 8-11). For instance, in a previous study, rosmarinic acid content of the methanol extract of *S. verticillata* ssp. *amasiaca* which was collected from Artvin was found as 24.1±1.67 µg/mg (11). Variation of rosmarinic acid amount among the species analyzed can be attributed to some certain factors, such as climate, harvesting time, and regional conditions.

CONCLUSION

Standardization is very important for the quality and safety of plant materials, so herein, rosmarinic, chlorogenic, and caffeic acid levels in *Salvia virgata*, *Salvia verticillata* ssp. *amasiaca* as well as five commercial *Salvia* tea bag samples were investigated using a previously validated HPLC-DAD method. Results of the study revealed that *Salvia* species are rich in rosmarinic acid and the commercial tea bag samples which belong to different firms have the same phenolic acid profile with significant differences in terms of rosmarinic acid content.

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