Determination of Rosmarinic Acid in Sage and Borage Leaves by High-Performance Liquid Chromatography with Different Detection Methods

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Abstract

Rosmarinic acid is separated and identified on the basis of high-performance liquid chromatography (HPLC)–UV–mass spectrometry data in 80% methanol in water extracts from the leaves of *Salvia* species (*S. officinalis, S. glutinosa, S. aethiopis, S. sclarea,* and *Borago officinalis*) as a dominant radical scavenger towards the 2,2'-diphenyl-1-picrylhydrazyl (DPPH•) stable radical in HPLC–DPPH• system. The content of rosmarinic acid in the plants is calibrated and quantitated from chromatograms obtained by UV detection at 280 nm. The concentration ranges from 13.3 to 47.3 mg of the phenolic acid per gram dried leaves of all plants is tested. *S. glutinosa* and *S. sclarea* have the highest concentration of rosmarinic acid. The amount of rosmarinic acid in borage leaves is similar compared with *Salvia officinalis* (15 mg/g). The HPLC–DPPH• system is calibrated for quantitative DPPH• scavenging assessment of rosmarinic acid. The results reveal excellent correlation (r^2 = 0.98) between the rosmarinic acid concentration and antiradical activity.

Introduction

Natural antioxidants are one of the compound groups that during last two decades attracted exceptionally high attention of researchers and health professionals. For instance, the hits generated from the advanced search of Science Citation Index expanded databases using the combination of the key words "natural" and "antioxidant" increased from 12 in 1990 to 328 and 284 in 2002 and 2003, respectively. A number of aromatic, spicy, and medicinal plants are known for their high content of antioxidants. Modern separation and detection techniques enabled scientists in comparatively short time to reveal a great number of new natural compounds possessing useful biological properties. Usually, the dominant antioxidants in herbs and spices are radical scavenging phenolic compounds, like flavonoids or phenolic acids. The development of analysis methods has played an important role in the rapid recent advances of antioxidant research.

Rosmarinic acid, as one of the most abundant and powerful natural antioxidant in various important *Lamiaceae* species, has been analyzed by using different detection and quantitative measurement methods. Most of these methods are based on chromatographic separation and further detection of target components. Thin-layer chromatography (TLC)–densitometry was used in several studies to determine rosmarinic and caffeic acids (1–4). In one of these studies, 96 *Lamiaceae* species were analyzed, and it was found that the concentration of rosmarinic acid ranged from 0.01 to 9.30 mg/g (4). The spectrophotometric method was used to directly determine rosmarinic acid in unpurified methanolic extracts by a complexation reaction with Fe^{2+} (5). Bonoli et al. reported fast separation and determination of carnosic and rosmarinic acids in rosemary extracts by capillary zone electrophoresis with UV–diode array detection (6). Rosmarinic acid enantiomers were separated and determined using three different chromatographic methods [high-performance liquid chromatography (HPLC), capillary electrophoresis, and gas chromatography] (7). Most recently, Fourier transform IR spectroscopy was reported as a new tool to determine rosmarinic acid in situ (8).

However, HPLC with spectrometric detectors has been most widely used for the separation, detection, and measurement of rosmarinic acid in plant extracts (9–12). In one such study, rosmarinic and caffeic acids were determined in rosemary, sage, thyme, spearmint, balm, and lavender; the concentration of rosmarinic acid varied from 2.0 to 27.4 mg/g (9). In more recent studies, there is a tendency to use a combination of more than one separation/detection/measurement method in order to increase the effectiveness of analysis of complex extracts usually containing a mixture of various components. Thus, the instrumental set consisting of liquid chromatography (LC)–UV detection–solid-phase extraction (SPE)–NMR apparatus–mass

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spectrometry (MS) was used for the online automated analysis of a Greek oregano (13) and *Rhaponticum carthamoides* (14) extracts.

However, none of the mentioned techniques provide information about radical scavenging/antioxidative properties of the separated and detected extract components. For this purpose, additional measurements are required. The use of reaction systems containing stable free radicals such as 2,2'-diphenyl-1-picrylhydrazyl (DPPH•) and 2,2'-azinobis (3-ethylbenzo- thiazoline-6-sulfonic acid) radical cation ($ABTS^{+\cdot}$) has proven to be a rapid and informative method for the assessment of crude extracts, purified fractions, and individual compounds. For instance, after comparing three methods, Koleva et al. concluded that being rapid, simple, and independent of sample polarity, the DPPH• method is very convenient for the quick screening of many samples (15).

Development of online detection of radical scavengers by HPLC–UV with extra luminol chemiluminescence (16), DPPH• (17), or $ABTS^{+\cdot}$ (18) detection modules was an important step in the research of antioxidatively active natural compounds. The methods were further improved (19, 20) and expanded by supplementing the online system with SPE and NMR instruments (21). The kinetics of the reaction of DPPH• with flavonoids were studied in detail by Butkovic at al. (22). During recent years, the online HPLC–DPPH•–ABTS• methods were succesfully used for the analysis of sweet grass (23), thyme (24), various *Salvia* species (25), borage (26), apples (27), coffee (28), *Geranium macrorrhizum* (29), *Potentila fruticosa* (30), *Mentha* species (31), and selected *Lamiaceae* species (20).

The present study is focused on the application of HPLC with UV, MS, and radical scavenging detectors for the analysis of antioxidative components and, particularly, rosmarinic acid in several *Salvia* species and borage leaves.

A great number of studies have shown garden sage to be a source of potent antioxidants (31–42). Antioxidant effects of sage were attributed to the main phenolic components, rosmarinic acid, caffeic acid derivative, and carnosic acid (34–36). Because of high antioxidative activity, *S. officinalis* extracts have been extensively used as a reference in the assessment of antioxidative properties of other, less-investigated plants (37–42). In previous studies, research interest has been focused mainly on the composition of active substances in *S. officinalis*. The other *Salvia* species (particularly *S. glutinosa*, *S. sclarea*, and *S. aethiopis*) were not studied so intensively. Malencicacute et al. found that rosmarinic acid was the dominant naturally occurring antioxidant in *S. reflexa* (43). Rosmarinic acid was also a major compound in *S. nemorosa* and *S. glutinosa,* as determined by TLC–densitometry, UV–vis–spectrometry, and LC–MS (44). Preliminary screening showed (24) that the extracts from various *Salvia* species demonstrate distinct antioxidative properties; therefore, it was of interest to obtain further information on their active components by applying recently developed methods.

Borage seed oil is of great interest among medical and nutritional research groups because of its high content of γ-linolenic acid. Wettasinge et al. (45) reported that rosmarinic, syringic, and sinapic acids are the major phenolic compounds in the ethanolic extract of borage seed meal. The extracts from borage leaves also demonstrated strong antioxidant activity (40); however, the information regarding their active components is very scarce.

Experimental

Extraction

The first-year vegetation plant leaves harvested in May were obtained from the collection of medicinal herbs at Kaunas Botanical Garden of Vytautas Magnus University (Kaunas, Lithuania). The solvents used were of analytical quality and purchased from Merck (Darmstadt, Germany). Rosmarinic acid was purchased from Roth (Karlsruhe, Germany), and DPPH• (95%) was purchased from Aldrich (Steinheim, Germany). The leaves were separated from other parts of the plants and dried at room temperature in the dark. Two grams of freshly crushed dry leaves were extracted with 100 mL 80% methanol in doubly distilled water using a homogenizer Büchi Mixer B-400 (Flawil, Switzerland). The homogenizer was switched on three times for 10 s with two short intervals for cooling the extraction mixture; the total extraction time was 30 s. The mixtures were filtered through paper filter MN 615 (Macherey-Nagel, Düren, Germany), and the resulting liquid extracts were stored in a freezer at –20°C under nitrogen until analysis. The analysis was performed within 1 month after the storage month.

HPLC–MS for identification of rosmarinic acid

HPLC analysis of the plant extracts was carried out on an HP 1100 LC equipped with a quaternary pump, thermostatted autosampler, diode array detector, and MS (Hewlett-Packard, Palo Alto, CA). For the separation, a LiChrospher RP-18 reversed-phase column (250- × 3-mm i.d., 5 µm) (Hewlett Packard) and a precolumn LiChroCART RP-18 (4- × 4-mm i.d.) was used. The column was eluted with 2% acetic acid in water as eluent A and acetonitrile as eluent B. The column was run with gradient elution at 0.3 mL/min (0–20 min 10–80% B, 20–25 min 80–100% B, and 25–30 min 100% B). The UV–absorption was monitored at 280 nm. The volume injected was 10 µL. MS analysis was performed using an HP quadrupole MS equipped with an electrospray interface. Drying nitrogen was heated to 350°C and the flow was 10 L/min. The capillary voltage was set to 4000 V, and the positive mode was used. For data acquisition, the MS operated over a mass range of *m/z* 100–450. For quantitation, the single ion monitoring (SIM) mode was used at *m/z* 383 for rosmarinic acid.

The methanolic extracts were used directly for HPLC–DPPH• analysis. The concentrations of reference substance rosmarinic acid used for the calibration curve was 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL.

HPLC–DPPH• online detection of radical scavengers

For detection of radical scavenging components, an online HPLC–DPPH• method was applied using a methanolic solution of DPPH• stable-free radical for post-column reaction (27). After HPLC separation and UV detection at 280 nm, the analytes reacted post column with the DPPH• at a concentration of 50 mg/mL in methanol. The radical absorbs at 515 nm, but upon reduction by an antioxidant or a radical species, the absorption

disappears. The deep violet DPPH• radical receives a proton from the antioxidants and is converted to a colorless protonated DPPH molecule. The flow of the reagent solution was set to 0.4 mL/min, and the induced radical solution bleaching is detected as a negative peak photometrically at 515 nm with a reaction time of 0.6 min.

The methanolic extracts were used directly for online HPLC–DPPH• analysis. The concentrations of rosmarinic acid used for calibration of the radical scavenging activity were 0.125, 0.25, 0.5, 0.75, and 1.00 mg/mL. Three replicates of each sample were measured, and the mean was calculated. The standard deviation was in the range of 1–5%.

Results and Discussion

HPLC analysis of the methanol extract of the *Salvia* species and borage resulted in the separation of rosmarinic acid as the major compound together with several minor components. Rosmarinic acid (Figure 1) was identified by comparison of the UV and mass spectra of the peaks separated by HPLC.

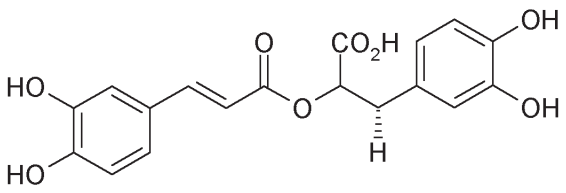

Figure 1. Chemical structure of rosmarinic acid.

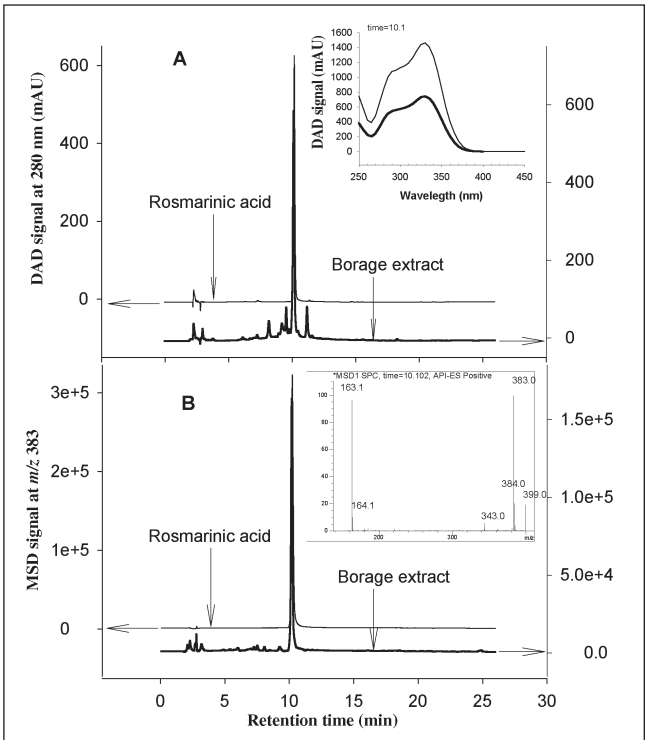

Figure 2. HPLC chromatograms with UV and mass spectra of rosmarinic acid in 80% methanol in water extract of borage. UV chromatogram at 280 nm with a UV spectrum in the wavelength range of 250–450 nm (A). Single ion mass chromatogram [*m/z* 383 = (rosmarinic acid + Na)$^+$] with a mass spectrum in the range of *m/z* 150–400 (B).

Absorption spectra and mass spectra that were obtained from HPLC of the borage extract are shown in Figure 2. For the *Salvia* extracts, similar chromatograms were obtained and rosmarinic acid was identified. As shown in Figure 2A, the major compound measured at 280 nm elutes after 10 min. This compound occurring in all plant extracts had an UV absorbance maximum at 330 nm and a minimum at 265 nm, with a characteristic shoulder at 290 nm. This could be attributed to a phenolic acid with two aromatic rings (46). Additionally, the shape of the UV spectra shows a very high accordance with rosmarinic acid. Figure 2B shows the intensity of the mass signal at 383 *m/z*. The mass spectrum of the peak (Figure 2B) shows the presence of the pseudo molecular ion associated with different cations *m/z* 399 $(M+K)^+$, 383 $(M+Na)^+$, and 361 $(M+H)^+$. The fragments that are similar in the standard and plant extracts comprise 343, 221, 185, 181, and 163. These results confirm the identity of rosmarinic acid.

The quantitative determination of rosmarinic acid in the plant extracts was achieved with a retention time of 10 min. The limit of detection (LOD) [signal-to-noise ratio (s/n) = 3] and limit of quantitation (LOQ) (s/n = 10) was 0.19 and 0.63 ng per injection for UV detection, respectively. The calibration curve of rosmarinic acid was linear over the ranges 25–500 µg/mL, with a correlation coefficient of r^2 = 0.9991. The content of rosmarinic acid in dried leaves of the sage species and borage was determined by the proposed method, and the results are shown in Table I. The concentration ranged from 13.3–47.3 mg of rosmarinic acid per gram dried leaves. *Salvia glutinosa* and *Salvia sclarea* had the highest concentration of rosmarinic acid. The amount of rosmarinic acid in borage leaves (15.0 mg/g) was in the same range as to other sages.

Radical scavenging activity of rosmarinic acid was carried out using the online HPLC–DPPH• method. Previously published reports showed that the method can be used for a rapid assessment of pure antioxidants and of antioxidative components in complex mixtures, particularly plant extracts (17,20,25). Simultaneously obtained UV and DPPH• radical quenching chromatograms using gradient elution of crude *S. officinalis, S. glutinosa, S. aethiopis*, *S. sclarea*, and *Borago officinalis* extracts isolated with 80% methanol in water are presented in Figure 3. HPLC analysis revealed the presence of several radical scavenging

Table I. Amount of Rosmarinic Acid in the Plants as Determined with HPLC–UV at 280 nm Using Gradient Elution

Plant name	Content (mg/g dry weight*) (*n* = 3)
Borago officinalis	15.0 ± 0.2
S. officinalis	19.5 ± 0.8
S. glutinosa	47.3 ± 2.1
S. sclarea	41.1 ± 1.9
S. aethiopis	13.3 ± 0.6

* The moisture was determined by distillation of 40 g of ground material with toluene.

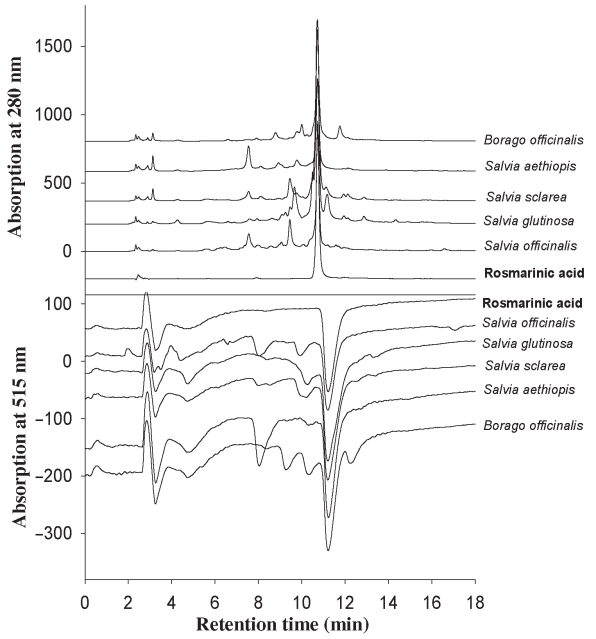

Figure 3. UV and DPPH• radical quenching chromatograms using elution of crude extracts of *S. officinalis*, *S. glutinosa*, *S. aethiopis*, *S. sclarea*, and *Borago officinalis*.

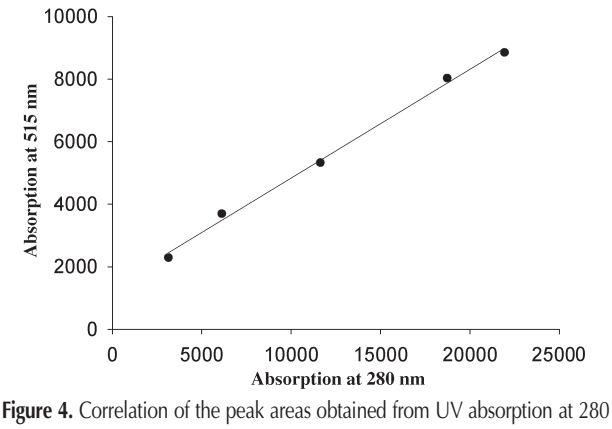

Figure 4. Correlation of the peak areas obtained from UV absorption at 280 nm and of negative peak areas obtained after postcolumn reaction with DPPH•.

components in all *Salvia* and borage extracts. However, rosmarinic acid in the extracts was the major radical scavenger obtained. The dependence of DPPH• reduction on the standard antioxidant concentration was tested by injecting five different concentrations ranging from 0.125 to 1 mg/mL of rosmarinic acid. A linear relationship between the negative peak areas of rosmarinic acid and injected concentrations was observed (r^2 = 0.9759). The LOD (s/n = 3) and LOQ (s/n = 10) for the negative peak of rosmarinic acid was calculated to be 15 and 48 ng per injection. A linear response (r^2 = 0.9962) was obtained between the negative and positive peak areas (Figure 4.). These results suggest that this method can be used for quantitating the antiradical activity of rosmarinic acid and other antioxidatively active components after HPLC separation.

Taking into account a wide range of the amount of important antioxidative components in plant material, which depend on species (4,9), variety, plant chemotype, cultivation and climatic conditions, harvesting time, and analysis method, the online HPLC–UV–MS–DPPH• method can be considered a promising techniques for quality control and extract standardization.

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References

1. G. Janicsak and I. Mathe. Parallel determination of rosmarinic and caffeic acids by TLC-densitometry. *Chromatographia* **46:** 322–24 (1997).
2. J. Nowakowska. Normal and reversed-phase TLC determination of rosmarinic and valerenic acids. *J. Planar Chromatogr.* **17:** 149–53 (2004).
3. I. Fecka, A. Kowalczyk, and W. Cisowski. Optimization of the separation of flavonoid glycosides and rosmarinic acid from *Mentha piperita* on HPTLC plates. *J. Planar Chromatogr.* **17:** 22–25 (2004).
4. G. Janicsak, I. Mathe, V. Miklossy-Vari, and G. Blunden. Comparative studies of the rosmarinic and caffeic acid contents of Lamiaceae species. *Biochem. Syst. Ecol.* **27:** 733–38 (1999).
5. T. Lopezarnaldos, M. Lopezserrano, A.R. Barcelo, A.A. Calderon, and J.M. Zapata. Spectrophotometric determination of rosmarinic acid in plant-cell cultures by complexation with Fe^{2+} ions. *Fresenius' J. Anal. Chem.* **351:** 311–14 (1995).
6. M. Bonoli, M. Pelillo, and G. Lercker. Fast separation and determination of carnosic acid and rosmarinic acid in different rosemary (*Rosmarinus officinalis*) extracts by capillary zone electrophoresis with ultra violet-diode array detection. *Chromatographia* **57:** 505–12 (2003).
7. A. Trute and A. Nahrstedt. Separation of rosmarinic acid enantiomers by three different chromatographic methods (HPLC, CE, GC) and the determination of rosmarinic acid in *Hedera helix* L. *Phytochem. Anal.* **7:** 204–208 (1996).
8. K. Stehfest, M. Boese, G. Kerns, A. Piry, and C. Wilhelm. Fourier transform infrared spectroscopy as a new tool to determine rosmarinic acid *in situ*. *J. Plant Physiol.* **161:** 151–56 (2004).
9. H.F. Wang, G.J. Provan, and K. Helliwell. Determination of rosmarinic acid and caffeic acid in aromatic herbs by HPLC. *Food Chem.* **87:** 307–11 (2004).
10. I. Parejo, F. Viladomat, J. Bastida, and C. Codina. Development and validation of a high-performance liquid chromatographic method for the analysis of antioxidative phenolic compounds in fennel using a narrow bore reversed phase C-18 column. *Anal. Chim. Acta* **512:** 271–80 (2004).
11. F.M. Areias, P. Valentao, P.B. Andrade, M.M. Moreira, J. Amaral, and R.M. Seabra. HPLC/DAD analysis of phenolic compounds from lavender and its application to quality control. *J. Liq. Chromatogr.* **23:** 2563–72 (2000).
12. M.F. Wang, R. Juliani, L. Zhang, Q.L. Wu, and J.E. Simon. "Analysis of flavonoids and rosmarinic acid contents in different varieties of mint by HPLC and LC-MS methods". In *Abstracts of Papers of the American Chemical Society*, vol. 226, U68-U68, 110-AGFD, Part 1, ACS, Washington, D.C., 2003.
13. V. Exarchou, M. Godejohann, T.A. van Beek, I.P. Gerothanassis, and J. Vervoort. LC-UV-solid-phase extraction-NMR-MS combined with a cryogenic flow probe and its application to the identification of compounds present in Greek oregano. *Anal. Chem.* **75:** 6288–94 (2003).

14. G. Miliauskas, T.A. van Beek, P. de Waard, and R.P. Venskutonis. "Identification of radical scavenging compounds in *Rhaponticum carthamoides* by means of LC-DAD-SPE-NMR". In *Future Trends in Phytochemistry, a Young Scientist Symposium*, Gargnano, Italy, 2004, pp. 29–30.
15. I.I. Koleva, T.A. van Beek, J.P.H. Linssen, A. de Groot, and L.N. Evstatieva. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochem. Anal.* **13:** 8–17 (2002).
16. A. Dapkevicius, T.A. van Beek, H.A.G. Niederlander, and A. de Groot. On-line detection of antioxidative activity in high-performance liquid chromatography eluates by chemiluminescence *Anal. Chem.* **71:** 736–40 (1999).
17. I.I. Koleva, H.A.G. Niederländer, and T.A. van Beek. An on-line HPLC method for detection of radical scavenging compounds in complex mixtures. *Anal. Chem.* **72:** 2323–28 (2000).
18. I.I. Koleva, H.A.G. Niederländer, and T.A. van Beek. Application of ABTS• radical cation for selective on-line detection of radical scavengers in HPLC eluates. *Anal. Chem.* **73:** 3373–81 (2001).
19. A. Dapkevicius, T.A. van Beek, and H.A.G. Niederlander. Evaluation and comparison of two improved techniques for the on-line detection of antioxidants in liquid chromatography eluates. *J. Chromatogr. A* **912:** 73–82 (2001).
20. M. Kosar, H.J.D. Dorman, O. Bachmayer, K.H.C. Baser, and R. Hiltunen. An improved on-line HPLC-DPPH• method for the screening of free radical scavenging compounds in water extracts of Lamiaceae plants. *Chem. Nat. Comp.* **39:** 161–66 (2003).
21. A. Pukalskas, T.A. van Beek, P. de Waard, and R.P. Venskutonis. "Development of a quintuple hyphenated HPLC-radical scavenging detection-DAD-SPE-NMR system for the rapid identification of antioxidants in complex plant extracts". In *Future Trends in Phytochemistry, a Young Scientist Symposium*, Gargnano, Italy, 2004, pp. 37–38.
22. V. Butkovic, L. Klasnic, and W. Bors. Kinetic study of flavonoid reactions with stable radicals. *J. Agric. Food Chem.* **52:** 2816–20 (2004).
23. A. Pukalskas, T.A. van Beek, R.P. Venskutonis, J.P.H. Linssen, A. van Veldhuizen, and A. de Groot. Identification of radical scavengers in sweet grass (*Hierochloe odorata*). *J. Agric. Food Chem.* **50:** 2914–19 (2002).
24. A. Dapkevicius, T.A. van Beek, G.P. Lelyveld, A. van Veldhuizen, A. de Groot, J.P.H. Linssen, and R. Venskutonis. Isolation and structure elucidation of radical scavengers from *Thymus vulgaris* leaves. *J. Nat. Prod.* **65:** 892–96 (2002).
25. D. Bandoniene, M. Murkovic, W. Pfannhauser, P.R. Venskutonis, and D. Gruzdiene. Detection and activity evaluation of radical scavenging compounds by using DPPH free radical and on-line HPLC-DPPH methods. *Eur. Food Res. Technol.* **214:** 143–47 (2002).
26. D. Bandoniene and M. Murkovic. The detection of radical scavenging compounds in crude extract of borage (*Borago officinalis* L.) by using an on-line HPLC-DPPH method. *J. Biochem. Biophys. Meth.* **53:** 45–49 (2002).
27. D. Bandoniene and M. Murkovic. On-line HPLC-DPPH screening method for evaluation of radical scavenging phenols extracted from apples (*Malus domestica* L.). *J. Agric. Food Chem.* **50:** 2482–87 (2002).
28. U. Svedstrom, K. Kahlos, A. Peltoketo, T. Nurmi, and R. Hiltunen. Determination of antiradical activity of coffee extracts by HPLC-DPPH method. *Eur. J. Pharm. Sci.* **19:** 29 (2003).
29. G. Miliauskas, T.A. van Beek, P.R. Venskutonis, J.P.H. Linssen, and P. de Waard. Antioxidative activity of *Geranium macrorrhizum*. *Eur. Food Res. Technol.* **218:** 253–61 (2004).
30. G. Miliauskas, T.A. van Beek, P.R. Venskutonis, J.P.H. Linssen, P. de Waard, and E.J.R. Sudhölter. Antioxidative activity of *Potentilla fruticosa*. *J. Sci. Food Agric.*, in press.
31. M. Kosar, H.J.D. Dorman, K.H.C. Baser, and R. Hiltunen. Screening of free radical scavenging compounds in water exracts of *Mentha* samples using a postcolumn derivatization method. *J. Agric. Food Chem.* **52:** 5004–10 (2004).
32. Y. Lu and L.Y. Foo. Flavonoid and phenolic glycosides from *Salvia officinalis*. *Phytochemistry* **55:** 263–67 (2000).
33. S.S. Chang, B. Ostric-Matijasevic, O.A.L. Hsieh, and C.-L. Huang. Natural antioxidants from rosemary and sage. *J. Food Sci.* **42:** 1102–1106 (1977).
34. M.-E. Cuvelier, H. Richard, and C. Berset. Antioxidant activity and phenolic composition of pilot-plant and commercial extracts or sage and rosemary. *J. AOCS* **73:** 645–52 (1996).
35. M. Wang, J. Li, M. Rangarajan, Y. Shao, E.J. La Voie, T.-C. Huang, and C.-T. Ho. Antioxidative phenolic compounds from sage (*Salvia officinalis*). *J. Agric. Food Chem.* **46:** 4869–73 (1998).
36. N. Okamura, Y. Fujimoto, S. Kuwabara, and A. Yagi. High-performance liquid chromatographic determination of carnosic acid and carnosol in *Rosmarinus officinalis* and *Salvia officinalis*. *J. Chromatogr. A* **679:** 381–86 (1994).
37. M.-E. Cuvelier, C. Berset, and H. Richard. Antioxidants in sage (*Salvia officinalis*). *J. Agric. Food Chem.* **42:** 665–69 (1994).
38. Y. Lu and L.Y. Foo. Rosmarinic acid derivatives from *Salvia officinalis*. *Phytochemistry* **51:** 91–94 (1999).
39. A. Dapkevicius, R. Venskutonis, T.A. van Beek, and J.P.H. Linssen. Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. *J. Sci. Food Agric.* **77:** 140–46 (1998).
40. K.G.C. Weel, P.R. Venskutonis, A. Pukalskas, D. Gruzdiene, and J.P.H. Linssen. Antioxidant activity of horehound (Marrubium vulgare L.) grown in Lithuania. *Fett/Lipid* **101:** 395–400 (1999).
41. D. Bandoniene, A. Pukalskas, P.R. Venskutonis, and D. Gruzdiene. Preliminary screening of antioxidant activity of some plant extracts in rapeseed oil. *Food Res. Int.* **33:** 785–91 (2000).
42. D. Bandoniene, D. Gruzdiene, and P.R. Venskutonis. Antioxidant activity of sage extracts in rapeseed oil treated with UV-rays. *Nahrung* **45:** 105–108 (2001).
43. V. Povilaityte and P.R. Venskutonis. Moldavian dragonhead (Dracocephalum moldavica L.) and Roman chamomile (Anthemis nobilis L.) extracts in rapeseed oil. *J. AOCS* **77:** 951–56 (2000).
44. D. Malencic, O. Gasic, M. Popovic, and P. Boza. Screening for antioxidant properties of Salvia reflexa Hornem. *Phytother. Res.* **14:** 546–48 (2000).
45. D.J. Malencic, M. Popovic, D. Stajner, N. Mimica-Dukic, P. Boza, and I. Mathe. Screening for antioxidant properties of Salvia nemorosa L. and Salvia glutinosa L. *Oxid. Commun.* **25:** 613–19 (2002).
46. M. Wettasinghe, F. Shahidi, R. Amarowicz, and M.M. Abou-Zaid. Phenolic acids in defatted seeds of borage (*Borago officinalis L.*). *Food Chem.* **75:** 49–56 (2001).

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