Available online at www.sciencedirect.com
SCIENCE DIRECT
Food Chemistry 100 (2007) 1283–1288
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Electrospray ionization mass spectrometry fingerprinting of essential oils: Spices from the Labiatae family

Jens K.S. Møller [a,*], Rodrigo R. Catharino [b], Marcos N. Eberlin [b]

[a] Royal Veterinary and Agricultural University, Department of Food Science, Food Chemistry, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark
[b] Thomson Mass Spectrometry Laboratory, Institute of Chemistry, State University of Campinas, UNICAMP, Campinas, SP 13083-970, Brazil

Received 21 July 2005; received in revised form 5 October 2005; accepted 23 October 2005

Abstract

Polar components of the methanolic extracts of the essential oils of the spices *Origanum dictamnus*, *Origanum vulgare*, *Origanum majorana* and *Rosmarinus officinalis*, all four belonging to the Labiatae family, were investigated by direct infusion electrospray ionisation mass spectrometry (ESI-MS) both in the negative and positive ion modes. Characteristic ESI mass spectra with many diagnostic ions were obtained for the extracts of all four spices, serving for fast and reliable identification of these species. Tandem mass spectrometry (ESI-MS/MS), which often forms a series of fragment ions, and this additional MS dimension increases selectivity for authenticity and adulteration tests for spice essential oils. The MS technique also provides complementary information of component structures revealing the presence of important bioactive components.

Keywords: Electrospray ionisation mass spectrometry fingerprinting; Labiatae family spices; Charged components; Natural antioxidants characterisation; Structural elucidation; Proof of authenticity

1. Introduction

Phenolic compounds from plants belong to a class of bioactive components that have received much attention during recent years, mainly owing to their positive effects on diet-health interaction in human nutrition (Rice-Evans, 2004). Industrial use of essential oils from plants for flavouring and fragrances in foods and cosmetics are also extensive and a market in continuing growth (Smith et al., 2005). The direct application of such natural herbal aromatic extracts for medical purposes is also well-known and becoming commonly accepted (Atoui, Mansouri, Boskou, & Kefalas, 2005; Pietta & Mauri, 2001; Smith et al., 2005; Sokmen et al., 2004). The beneficial effects of components present in essential oils have been described since ancient times and pain relieving effects or prevention against inflections are effects often attributed to plant and herb extracts. However, as products derived from such plant and herbal extracts are used as dietary supplements or for direct medical purposes, it is crucial to screen for their complete composition and to ensure authenticity and product quality as falsification may cause life-threatening poisoning if toxic adulterants or substitutes are used.

The ability of essential oils to act as antioxidants both in vivo in human biology (Spencer, El Mohsen, & Rice-Evans, 2004; Williams, Spencer, & Rice-Evans, 2004), and in vitro in foods (Zandi & Ahmadi, 2000), cosmetics or pharmaceuticals (Moure et al., 2001) is an area of crescent scientific investigations. A recent review has evaluated major studies on natural antioxidants, and the performance of model systems and physiological studies (Becker, Nissen, & Skibsted, 2004) suggesting that preliminary studies should focus on chemical identification and characterisation of the potential antioxidants. Most chemical screenings of plant extracts employ gas chromatography coupled to mass spectrometry (GC–MS), thereby measuring mostly the more volatile components (Sherma, 2003).

* Corresponding author. Tel.: +45 3528 32 90; fax: +45 3528 33 44.
E-mail address: jemo@kvl.dk (J.K.S. Møller).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.10.013

The application of alternative techniques such as electrospray ionisation mass spectrometry (ESI-MS) fingerprinting to screen medical plants is rare (Mauri & Pietta, 2000). ESI-MS proves very fast and versatile employing little sample preparation to yield immediate compositional information of the most polar ESI-ionisable compounds. These unique features of direct infusion ESI-MS have recently been applied for fingerprinting of complex mixtures such as bee propolis (Sawaya et al., 2004), beer (Araujo et al., 2005), wine (Cooper & Marshall, 2001), whisky (Møller, Catharino, & Eberlin, 2005), vegetable oil (Wu, Rodgers, & Marshall, 2004) and crude oil (Rodgers, Schaub, & Marshall, 2005).

We report herein direct infusion ESI-MS fingerprint characterization of methanolic extracts of four different spices from the Labiatae family. We used these four spices as illustrative examples to demonstrate that ESI-MS fingerprints both in the positive and negative ion modes offer an attractive complementary technique (mainly for GC–MS) for broad-range studies of the more polar bioactive components. ESI-MS fingerprinting is also shown to be able to characterize each particular spices via their essential oils or aroma extracts. The use of ESI-MS/MS on diagnostic ions adds a second dimension of mass and structure analysis enabling even more secure spice fingerprint screenings and often structural assignments.

2. Materials and methods

2.1. Chemicals

HPLC-grade methanol, ammonium hydroxide and formic acid solutions were purchased from Sigma-Aldrich and used with no further purification.

2.2. Materials

Dried leaves of Dittany (*Origanum dictamnus* L.) were purchased locally at the farmers market in Hania on Crete. Dried leaves of Turkish oregano (*Origanum vulgare* L.), Sweet Marjoram (*Origanum majorana* L.) and Rosemary (*Rosmarinus officinalis* L.) were obtained as commercial available samples from a local supermarket.

2.3. Spice extraction

Extraction of the spices was carried out as previously described yielding a final spice amount of 0.25 mg mL^{-1} extraction solvent (Møller, Madsen, Aaltonen, & Skibsted, 1999). The methanol extracts were kept in brown flasks at 5 °C until analysis, which was carried out within a few hours after preparation.

2.4. Electrospray ionization mass spectrometry

A Q-TOF mass spectrometer (Micromass, Manchester, UK) was used for fingerprinting ESI-MS analysis. The general conditions were: source temperature of 100 °C, capillary voltage of 2.1 kV and cone voltage of 40 V. For measurements in the negative ion mode, ESI(−) MS, 10.0 μl of concentrated NH_4OH aqueous solution was added to the sample mixture to a total volume of 1000 μl yielding 0.1% as final concentration. Likewise, 10.0 μl of concentrated aqueous formic acid solution was added for ESI(+)-MS giving a final concentration of 0.1%. ESI-MS was performed by direct infusion with a flow rate of 10 μl min^{-1} using a syringe pump (Harvard Apparatus). Mass spectra were acquired and accumulated over 60 s and spectra were scanned in the range between 50 and 1000 *m*/*z*.

2.5. Tandem mass spectrometry

Structural analysis of single ions in the mass spectra from spice extracts was performed by mass-selecting the ion of interest, which was in turn submitted to 15–55 eV collisions with argon in the collision quadrupole. The collision gas pressure was optimized to produce extensive fragmentation of the ion under investigation.

2.6. Data handling

All data obtained from ESI-MS of the various spice extracts were treated using MassLynx 3.5 (Waters, Manchester, UK). Mass spectral data was accumulated over approximately 20 s. and the relevant mass range was selected and enlarged, which depending on the ESI-MS mode varied between *m*/*z* 50 and 550 or 900, respectively. These ranges contained the great majority of the ions of interest as judged by visual inspection.

3. Results and discussion

The methanol extract of the three oregano variants Dittany, Turkish oregano, and Sweet Marjoram all have clear greenish colour, with varying intensity, whereas the Rosemary extract has a distinct brown colour. This colour difference can result from the use of different parts of the plants to form the dried spices. For instance, the spices giving the greenish extracts are only constituted of leaf parts, while Rosemary that gives the brownish extract the parts used seem quite different in composition and probably contain relatively more of other components of lignin or wood origin.

ESI(+)-MS: Fig. 1 shows the ESI(+)-MS of the four spice extracts. It is evident that all four extracts contain numerous polar components that form upon ESI a relatively complex and considerably distinct set of likely protonated and cationized (Na^+ and K^+) molecules mainly up to *m*/*z* 800. Those for Sweet Marjoram, Turkish oregano and Dittany share some common ions mainly those of *m*/*z* 104, 219, and 871. The Sweet Marjoram extract is characterized mainly by the ions of *m*/*z* 203 and 399, while showing the ion of *m*/*z* 219 as far the most abundant. The

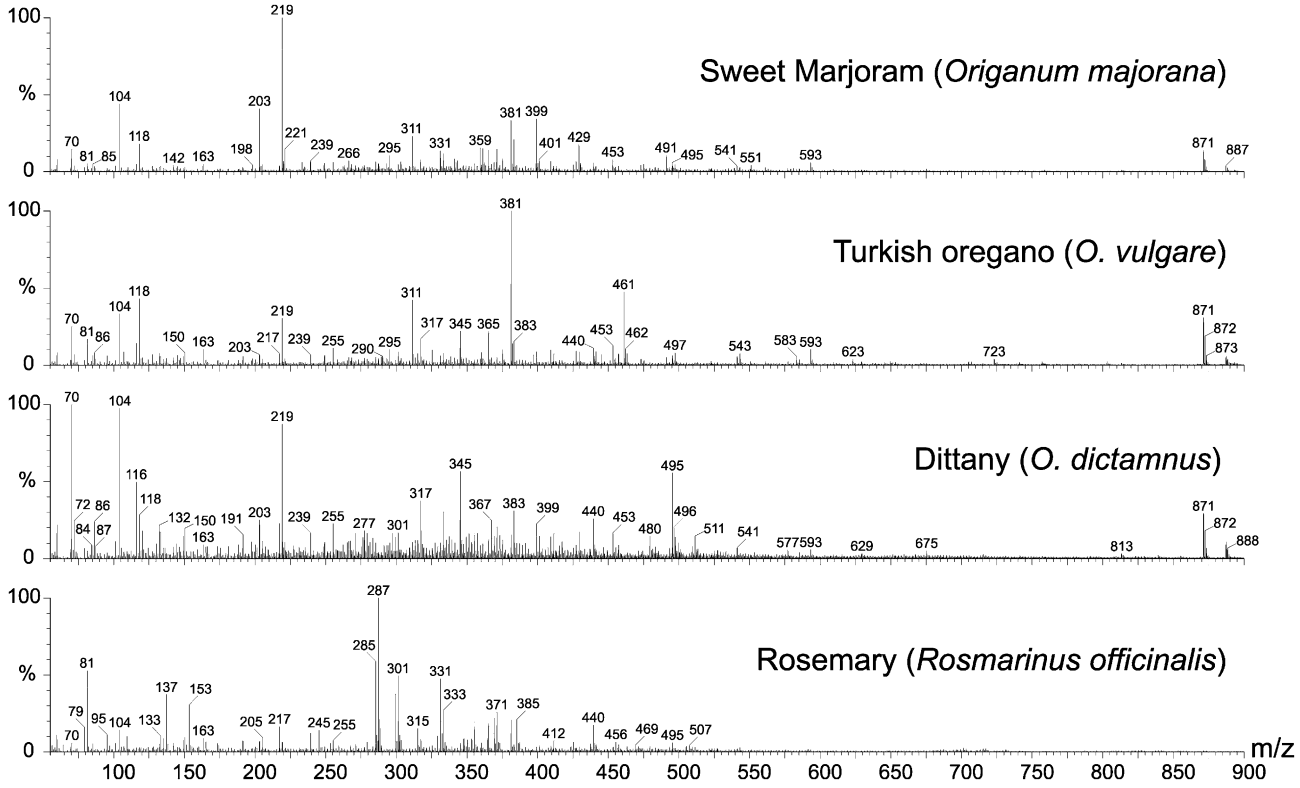

Fig. 1. ESI(+)-MS fingerprints of methanolic extracts containing 0.25 g spice per ml of four spices from the Labiatae family.

Turkish oregano extract is mainly characterized by the abundant ions of *m*/*z* 381 and 461, whereas the ions of 345 and 495 are the main diagnostic ions for Dittany. The Rosemary extract displays a unique ESI(+)-MS with several diagnostic cations, most notably those of *m*/*z* 287, 285, 137, and 153.

ESI-MS/MS of the majority of these diagnostic cations show limited dissociation, but some show rich and characteristic distribution of fragment ions as Fig. 2 illustrates for that of *m*/*z* 287 from the extract of Rosemary. Protonated kaempferol or other isomeric $C_6C_3C_6$ flavonoids with four hydroxyl groups are likely candidates for the ion of *m*/*z* 287 as they have MW of 286 Da. Previous MS/MS studies of flavonoid aglycones have shown that they dissociate by breaking of the two C–C bonds in the central C ring followed by a retro-Diels-Alder reaction as illustrated in Fig. 3 (Cuyckens & Claeys, 2004). However, the presently observed MS/MS fragmentation pattern cannot exactly match product ions containing intact A and B rings as have previously been reported (Ma, Li, Van den Heuvel, & Claeys, 1997).

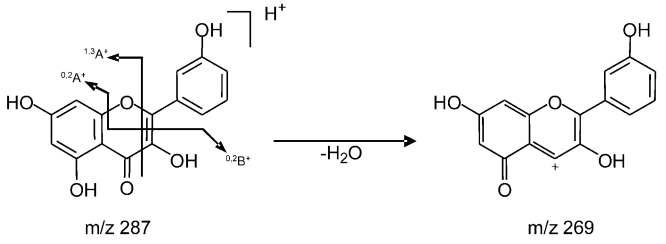

Fig. 3. Ion products formed from protonated flavonol, e.g. kaempferol, by CID yielding positively charged, intact A and B ring (cleavage at position 0–3 in ring C) and dehydrated fragments.

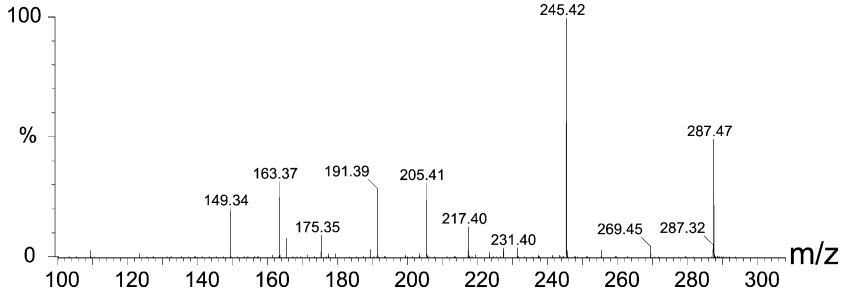

Fig. 2. ESI(+)-MS/MS of the cation of *m*/*z* 287.4 from the extract of Rosemary. Collision energy of 35 V.

A distinct feature within the ESI(+)-MS of the four spice extracts is that Sweet Marjoram, Turkish oregano, and Dittany exhibit a number of significant cations in the m/z 500–900 range particularly that of m/z 871, whereas these ions are not observed in the Rosemary extract. The component forming the ion of m/z 871 may be related to the greenish colour of the extracts. For instance, chlorophyllide *a*, which is a derivative of chlorophyll *a*, consists of a protoporphyrin ring and a long chained phytol alcohol, while the central Mg metal is absent. This compound has the molecular formula $C_{55}H_{74}N_4O_5$ (870 Da) whose protonated molecule therefore corresponds in mass and isotopic pattern to the ion of m/z 871.

ESI(−)-MS: Fig. 4 shows the ESI(−)-MS fingerprints for the methanol extracts of the four spices. Unique fingerprints with several diagnostic anions mainly of $m/z < 500$ are obtained for each of the four spice extracts. Sweet Marjoram and Turkish oregano extracts yield a much richer variety of anions as compared to Dittany and Rosemary. It is also possible to pinpoint several major diagnostic

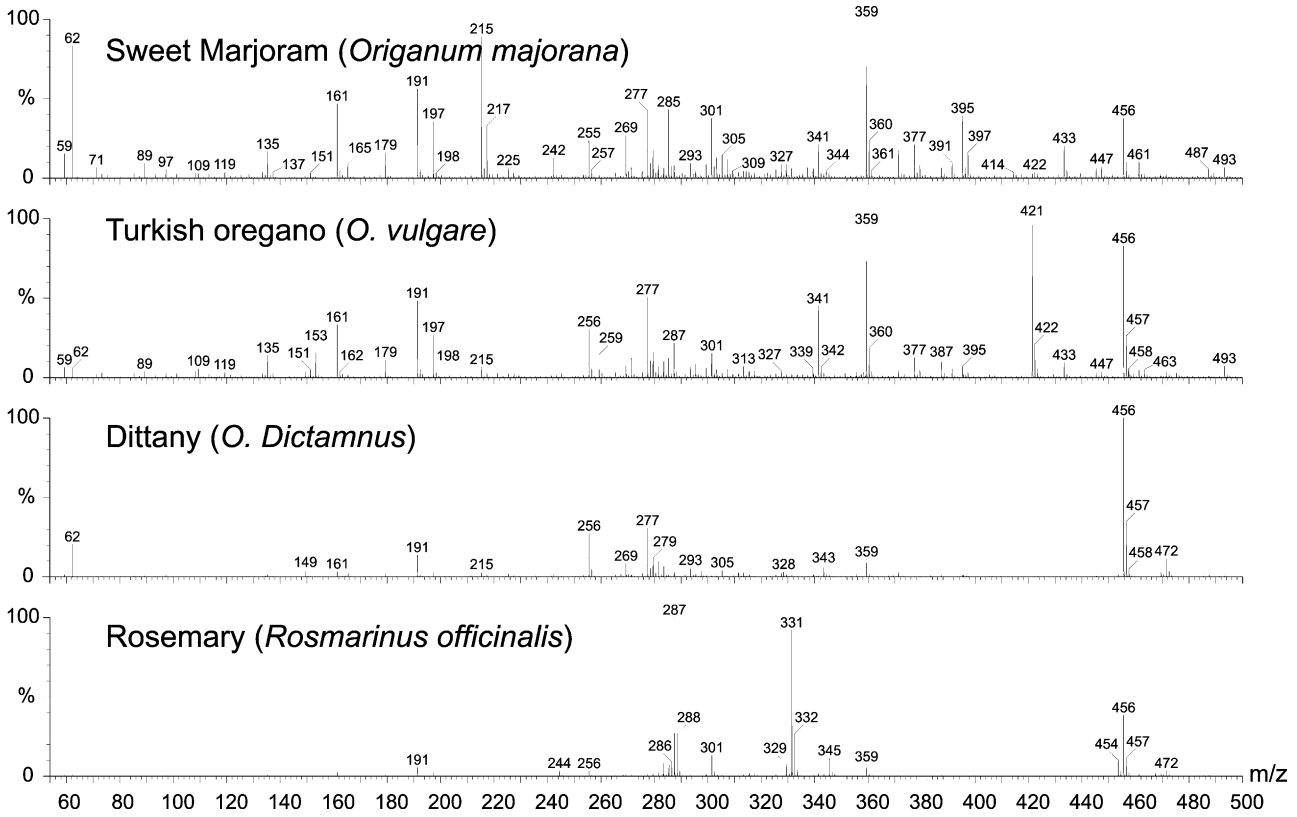

Fig. 4. ESI(−)-MS fingerprints of methanolic extracts containing 0.25 g per ml of four spices from the Labiatae family.

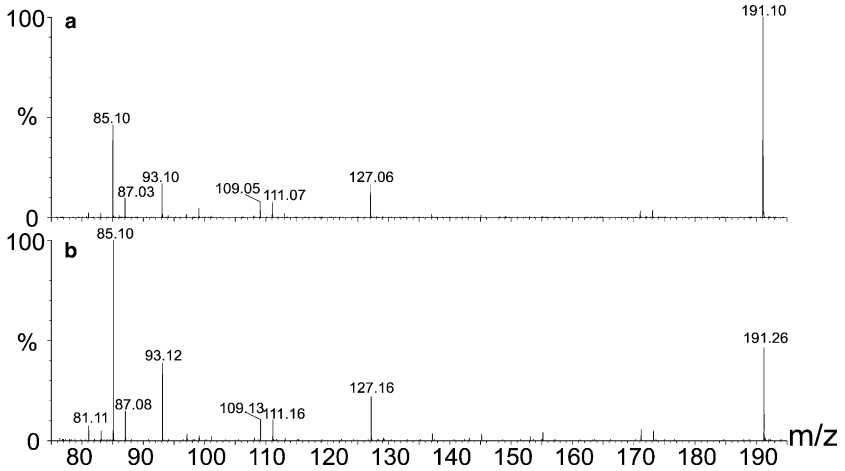

Fig. 5. ESI(+)-MS/MS of ion of m/z 191 from the methanolic extracts of (a) Dittany and (b) Turkish oregano. Collision energy of 20 V. The corresponding spectra for both the Rosemary and Marjoram extracts were nearly identical.

anions, the most relevant being those of *m*/*z* 215 and 217 for Sweet Marjoram; *m*/*z* 421 for Turkish oregano; and *m*/*z* 331 for Rosemary. For Dittany, no prominent diagnostic anion is detected, but the far most abundant anion being that of *m*/*z* 456 is a very characteristic feature of this spice extract.

The ESI(−)-MS/MS data provide valuable information. For instance, ion of *m*/*z* 191 dissociates to a set of fragment ions that match those of deprotonated quinic acid, which can be exemplified by the two nearly identical ESI-MS/MS collected for this anion from Dittany and Turkish oregano extracts (Fig. 5). Furthermore, ESI(−)-MS/MS of the ion of *m*/*z* 359 detected in varying abundances in all four spice extracts are nearly identical, as Fig. 6 illustrates for Rosemary and Sweet Marjoram. These ESI(−)-MS/MS correspond well with that of deprotonated rosmarinic acid, as judged by the formed ion fragments, as these are compatible with the two molecular parts constituting this compound, i.e. the deprotonated form of caffeic acid and the 2-hydroxy derivative of hydrocaffeic acid, respectively, plus dehydrated ion fragments of these compounds as shown in the reaction scheme in Fig. 7. Previously, rosmarinic acid has been identified in extracts of the following spices; Dittany, Turkish Oregano and Sweet Marjoram (Triantaphyllou, Blekas, & Boskou, 2001), and this phenolic compound is likely to be present in relatively high proportions in extracts of Turkish oregano (Capecka, Mareczek, & Leja, 2005).

As these ESI-MS fingerprints in both positive and negative ion mode clearly demonstrate, polar components are likely to provide straightforward discrimination between spice extracts, thereby reflecting their varying content of many bioactive compounds, such as flavonoids, polyphenolic acids and anthocyanins. Phenolic compounds present in plants constitute a complex mixture, and so far only a limited number of plants have been investigated systematically for their phenol content and these studies have only to a limited extent employed the analytical technique of ESI-MS (Robards, 2003). Direct infusion ESI-MS screening seems therefore very promising for fast broad-range investigation of phenolic compounds in diverse plant extracts.

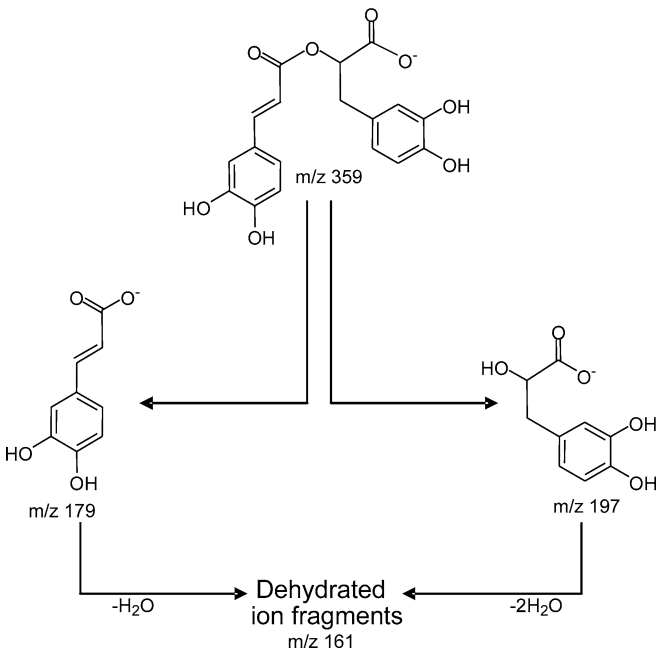

Fig. 7. Ion products formed from deprotonated rosmarinic acid by CID yielding two principal components (*m*/*z* 197 and 179) and also dehydrated ion fragments with *m*/*z* 161.

Since GC–MS analysis of essential oils has naturally focused its attention to the many volatile terpenes present in such mixtures, the fast fingerprint characterization by ESI-MS can also reveal distributions of other important classes of compounds not always accessed via GC–MS owing to lack of or reduced volatility (van Breemer, Farnsworth, Fong, & Pauli, 2005). This more comprehensive compositional screening of spice extracts offered by ESI-MS fingerprinting should in the future facilitate identification and authentication of plants and herbs, which normally require employment of multidisciplinary efforts within pharmacognosy, analytical chemistry and molecular biology (van Breemer et al., 2005).

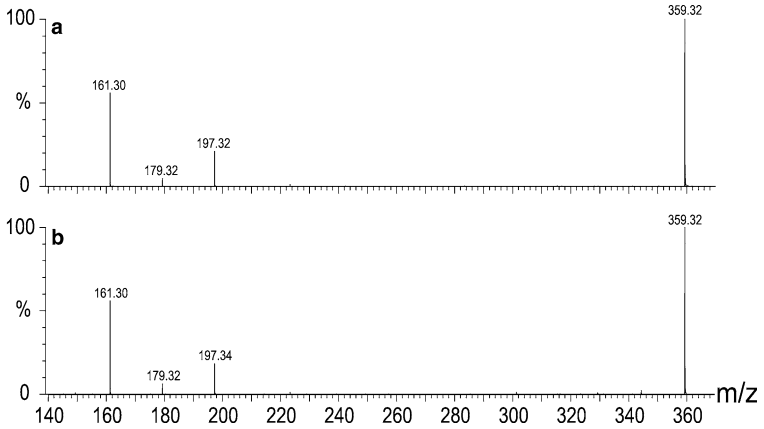

Fig. 6. ESI(+)-MS/MS of ion of *m*/*z* 359.4 from the methanolic extracts of (a) Rosemary and (b) Sweet Marjoram. Collision energy was 15 V. The corresponding spectra for both Dittany and Turkish oregano extracts were nearly identical.

Acknowledgements

Jens K.S. Møller is grateful to the International Committee of the Royal Veterinary and Agricultural University, Denmark, for the financial support during his stay in Brazil. Professor L.H. Skibsted is kindly thanked for providing the Dittany spice, which was purchased at the local market on Crete.

References

Araujo, A. S., da Rocha, L. L., Tomazela, D. M., Sawaya, A. C. H. F., Almeida, R. R., Catharino, R. R., et al. (2005). Electrospray ionization mass spectrometry fingerprinting of beer. *Analyst, 130*(6), 884–889.

Atoui, A. K., Mansouri, A., Boskou, G., & Kefalas, P. (2005). Tea and herbal infusions: Their antioxidant activity and phenolic profile. *Food Chemistry, 89*(1), 27–36.

Becker, E. M., Nissen, L. R., & Skibsted, L. H. (2004). Antioxidant evaluation protocols: Food quality or health effects. *European Food Research and Technology, 219*(6), 561–571.

Capecka, E., Mareczek, A., & Leja, M. (2005). Antioxidant activity of fresh and dry herbs of some Lamiaceae species. *Food Chemistry, 93*(2), 223–226.

Cooper, H. J., & Marshall, A. G. (2001). Electrospray ionization Fourier transform mass spectrometric analysis of wine. *Journal of Agricultural and Food Chemistry, 49*(12), 5710–5718.

Cuyckens, F., & Claeys, M. (2004). Mass spectrometry in the structural analysis of flavonoids. *Journal of Mass Spectrometry, 39*(1), 1–15.

Ma, Y. L., Li, Q. M., Van den Heuvel, H., & Claeys, M. (1997). Characterization of flavone and flavonol aglycones by collision-induced dissociation tandem mass spectrometry. *Rapid Communications in Mass Spectrometry, 11*(12), 1357–1364.

Mauri, P., & Pietta, P. (2000). Electrospray characterization of selected medicinal plant extracts. *Journal of Pharmaceutical and Biomedical Analysis, 23*(1), 61–68.

Møller, J. K. S., Catharino, R. R., & Eberlin, M. N. (2005). Electrospray ionisation mass spectrometry fingerprinting of whisky: Immediate proof of origin and authenticity. *Analyst, 130*(6), 890–897.

Møller, J. K. S., Madsen, H. L., Aaltonen, T., & Skibsted, L. H. (1999). Dittany (*Origanum dictamnus*) as a source of water-extractable antioxidants. *Food Chemistry, 64*(2), 215–219.

Moure, A., Cruz, J. M., Franco, D., Dominguez, J. M., Sineiro, J., Dominguez, H., et al. (2001). Natural antioxidants from residual sources. *Food Chemistry, 72*(2), 145–171.

Pietta, P., & Mauri, P. (2001). Analysis of flavonoids in medicinal plants. *Methods in enzymology, 335*, 26–45.

Rice-Evans, C. (2004). Flavonoids and isoflavones: absorption, metabolism, and bioactivity. *Free Radical Biology and Medicine, 36*(7), 827–828.

Robards, K. (2003). Strategies for the determination of bioactive phenols in plants, fruit and vegetables. *Journal of Chromatography A, 1000*(1-2), 657–691.

Rodgers, R. P., Schaub, T. M., & Marshall, A. G. (2005). Petroleomics: MS returns to its roots. *Analytical Chemistry, 77*(1), 20A–27A.

Sawaya, A. C. H. F., Tomazela, D. M., Cunha, I. B. S., Bankova, V. S., Marcucci, M. C., Custodio, A. R., et al. (2004). Electrospray ionization mass spectrometry fingerprinting of propolis. *Analyst, 129*(8), 739–744.

Sherma, J. (2003). High-performance liquid chromatography/mass spectrometry analysis of botanical medicines and dietary supplements: A review. *Journal of Aoac International, 86*(5), 873–881.

Smith, R. L., Cohen, S. M., Doull, J., Feron, V. J., Goodman, J. I., Marnett, L. J., et al. (2005). A procedure for the safety evaluation of natural flavor complexes used as ingredients in food: essential oils. *Food and Chemical Toxicology, 43*(3), 345–363.

Sokmen, M., Serkedjieva, J., Daferera, D., Gulluce, M., Polissiou, M., Tepe, B., et al. (2004). In vitro antioxidant, antimicrobial, and antiviral activities of the essential oil and various extracts from herbal parts and callus cultures of *Origanum acutidens*. *Journal of Agricultural and Food Chemistry, 52*(11), 3309–3312.

Spencer, J. P. E., El Mohsen, M. M. A., & Rice-Evans, C. (2004). Cellular uptake and metabolism of flavonoids and their metabolites: implications for their bioactivity. *Archives of Biochemistry and Biophysics, 423*(1), 148–161.

Triantaphyllou, K., Blekas, G., & Boskou, D. (2001). Antioxidative properties of water extracts obtained from herbs of the species Lamiaceae. *International Journal of Food Sciences and Nutrition, 52*(4), 313–317.

van Breemer, R.B., Farnsworth, N.R., Fong, H.S., Pauli, G.(2005). Authentication of herbs and herbal products. 229th ACS National Meeting, San Diego, California, March 13–17.

Williams, R. J., Spencer, J. P. E., & Rice-Evans, C. (2004). Flavonoids: antioxidants or signalling molecules? *Free Radical Biology and Medicine, 36*(7), 838–849.

Wu, Z. G., Rodgers, R. P., & Marshall, A. G. (2004). Characterization of vegetable oils: detailed compositional fingerprints derived from electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *Journal of Agricultural and Food Chemistry, 52*(17), 5322–5328.

Zandi, P., & Ahmadi, L. (2000). Antioxidant effect of plant extracts of Labiatae family. *Journal of Food Science and Technology-Mysore, 37*(4), 436–439.