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Electrospray characterization of selected medicinal plant extracts

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Abstract

Extracts of selected medicinal plants were examined by electrospray mass spectrometry (ESI-MS). This technique allowed identification of the main components of each extract, thereby providing a typical finger-print of the examined plants. More specifically, anthocyanins (*Vaccinium myrtillus*), isoflavones (*Glycine max*, soybean), flavonol-glycosides and terpenes (*Ginkgo biloba*), triterpenes (*Centella asiatica*), caffeoyl-quinic acids (*Cynara scolymus*, artichoke), ginsenosides (*Panax ginseng*), catechins (*Camellia sinensis*, green tea) and flavones and flavanones (Propolis) were detected rapidly at levels in the range of 0.1–1 μg/ml, using 0.2–1 mg/ml of each medicinal plant extract. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Medicinal plants; Electrospray mass spectrometry; Finger-print; Flavonoids; Terpenes

1. Introduction

Medicinal plants continue to draw wide attention for their role in case of mild/chronic diseases and herbal medicines have received an increasing interest as documented by the numerous and rigorous studies published.

A number of medicinal plants have been proved to offer an alternative to synthetic drug substances in preventing and treating some chronic and mild diseases, providing they are of adequate quality and properly used. Many factors influence the quality of herbs, including species variation, environmental conditions, time of harvesting, storage and processing. In addition, herbal extracts may be added with other plants devoid or exhausted of active constituents. For these reasons, the quality control of herb standardized extracts is an essential part of any research involving safety, efficacy and therapeutical reproducibility. Quality control is not easy, because medicinal extracts are a complex mixtures of different compounds and often their identity is only partially known.

Among the active principles present in medicinal plants, flavonoids, terpenes and caffeic acid derivatives attracted a great interest [1–5].

These compounds have been analyzed by means of gas-chromatography coupled to mass spec-

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trometry (GC-MS) [6], high-performance liquid chromatography (HPLC) [1,2] and capillary electrophoresis (CE) [3,4]. HPLC and CE allow an efficient separation of flavonoids in different plant extracts. Moreover, it is possible to identify the compounds present in the extracts combining chromatographic and electrophoretic results with the on-line UV spectra achieved by diode array detection (DAD) [2].

In the past years, thermospray mass spectrometry (TSP-MS) was also applied to the analysis of flavonoids and terpenes in plant extracts, such as *Ginkgo biloba* [7] and *Hypericum perforatum* [8]. Unfortunately, TSP-MS methodology is not indicated for thermolabile compounds. For example, flavonol-glycosides analyzed by TSP-MS produce mainly aglycone fragments, while the molecular ions $[MH]^+$ are present at very low abundance [9,10].

On the contrary, electrospray ionization mass spectrometry (ESI-MS) allows a softer ionization, and permits to obtain structural information using collisionally induced dissociation (CID). Moreover, ESI-MS makes possible to discriminate between a various flavonoid classes and to provide information on the glycosylation position [11].

Due to the absence (or low level) of fragmentation, the electrospray technique is suitable to characterize not only single compounds, but also complex mixtures, like those of herbal extracts. This means that a rapid screening of molecular species present in an extract may be performed without chromatographic separation.

Therefore, different plant extracts were analyzed by direct infusion into ESI-MS apparatus, with the aim to obtain their finger-prints. The extracts are representative of some important classes, including anthocyanins (*Vaccinium myrtillus*), isoflavones (*Glycine max*, soybean), flavonol-glycosides and terpenes (*G. biloba*), triterpenes (*Centella asiatica*), caffeoyl-quinic acids (*Cynara scolymus*, artichoke), ginsenosides (*Panax ginseng*), catechins (*Camellia sinensis*, green tea), and flavones and flavanones (Propolis).

2. Experimental

2.1. Chemicals

Commercial standards were obtained from Extrasynthese (Genay, France). Dry extracts were supplied from Indena S.p.A. (Milan, Italy) and Specchiasol s.r.l. (Bussolengo, Italy).

All other reagents were HPLC grade (J.T. Baker, Deventer, Holland).

2.2. Sample preparation

Extracts were dissolved in methanol. Extract concentrations were in the range of 0.5–5 mg/ml.

2.3. Mass spectrometry

Electrospray mass spectrometric analyses were performed on a Hewlett-Packard 5989A equipped with an electrospray interface 59987A. Nitrogen was used as nebulizing gas at a pressure of 50 psi and a temperature of 300°C.

Direct infusion conditions: the samples were analyzed by direct infusion in ESI-MS by means of a syringe pump (Harvard Apparatus, Natick, MA) at flow-rate of 10 μl/min in scan mode.

3. Results and discussion

Each plant extract was analyzed by direct infusion in ESI-MS at a flow-rate of 10 μl/min. The extracts were dissolved in water:methanol (50/50) and centrifuged before injection in mass spectrometer. Mass spectra were acquired in scan mode detection and ESI-MS conditions were optimized using available standard for each class of compounds. Some examples of the obtained results are reported below.

3.1. Vaccinium myrtillus

V. myrtillus is characterized by the presence of anthocyanins and their aglycones (anthocyanidins), which are used mainly in ophthalmic affections [12].

A typical ESI-MS spectrum of *V. myrtillus* obtained in positive detection is shown in Fig. 1. The most abundant ions are due to the molecular ions of anthocyanidins. In particular, the ions with m/z 287, 303, 317 and 331 are related to cyanidin, delphinidin, petunidin and malvidin, respectively. In addition the glycosides derivatives are also present as molecular ions: m/z 419, 449, 463, 479 and 493 correspond to cyanidin-3-O-arabinoside, cyanidin-3-O-glucoside/petunidin-3-O-arabinoside, delphinidin-3-O-glucoside/malvidin-3-O-arabinoside, petunidin-3-O-glucoside and malvidin-3-O-glucoside, respectively. Of course, using ESI-MS it was not possible to distinguish glucoside from galactoside derivatives. In this way, positive ESI-MS permitted to detect simultaneously 9 different ions related to anthocyanidins and anthocyanins.

The ESI-MS finger-print of *V. myrtillus* extracts is in good agreement with the results reported by Morazzoni et al. [13], but it differs significantly from the finger-print obtained for *Catharanthus roseus* [14]. This mean that it is possible by a simple ESI-MS spectrum to differentiate two extracts containing the same class of compounds.

These compounds showed only the molecular ions, with no sodium or potassium adducts, due to the absence of a carbonyl group in position 4. In fact, the formation of alkali ion adducts requires glycosylation in position 3 together with the presence of a carbonyl group in position 4, such as for flavonol-3-O-glycosides. This structure allows the formation of a crown-embedded cation (Na^+ or K^+) [11].

3.2. Panax ginseng

P. ginseng is widely used in herbal medicine, and it is characterized for its content of ginsenosides, which have been shown to produce favourable effects in human performance [15]. The presence of ginsenosides in *P. ginseng* extracts was detected by means of direct infusion in electrospray. ESI-MS conditions were first optimized in positive mode using ginsenosides R_{b1} and R_{g1} as reference standards. Then *P. ginseng* extract was analyzed, and a typical mass spectrum is shown in Fig. 2. Seven ginsenosides were detected as potassium adducts: m/z 1147 correspond to

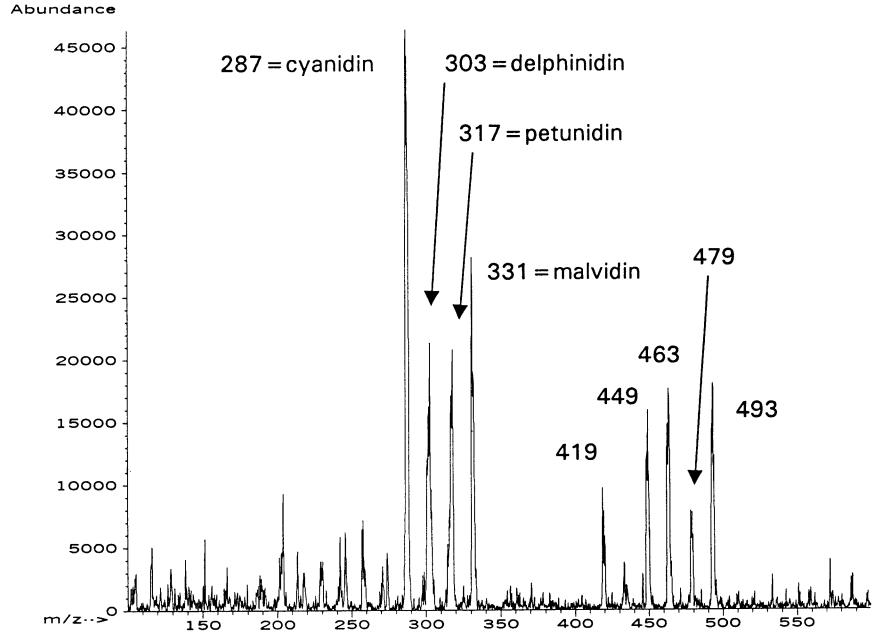

Fig. 1. Typical positive mass spectrum of *Vaccinium myrtillus* extract.

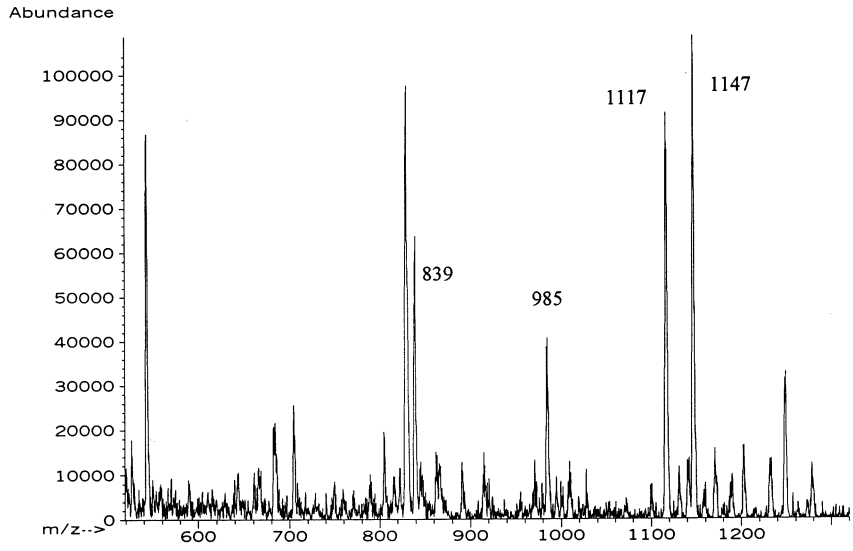

Fig. 2. Typical positive mass spectrum of *Panax ginseng* extract.

ginsenoside R_{b1}; ion m/z 1117 is related to different isomers, R_{b2} and R_c. In the same way, the ions m/z 985 and 839 correspond to the pairs of isomers R_d/R_e and R_{g1}/R_f, respectively.

When a phytosome preparation of *P. ginseng* was analyzed, the corresponding ESI-MS spectrum changed. In this case, the main ions m/z 823 and 801 correspond to the sodium adduct ($[M+Na]^+$) and molecular ion ($[M+H]^+$) of ginsenosides R_{g1} and R_f. Molecular ions of ginsenosides R_d and R_e (m/z 947) are present at lower abundance. On the contrary, the ginsenosides R_{b1}, R_{b2} and R_c cannot be evidenced. It is likely that these derivatives are tightly complexed, and are not detectable under our experimental conditions.

3.3. Centella asiatica

Triterpene saponins (madecassoside and asiaticoside) and their sapogenins (madecassic and asiatic acid) are regarded as the active principles in *C. asiatica* [16]. Fig. 3 shows a negative ESI-MS mass spectrum of *C. asiatica* extract. The main ions m/z 488 and 504 correspond to asiatic acid and madecassic acid, respectively. In addition, asiaticoside (m/z 957) and madecassoside (m/z 974) were detected.

3.4. Camellia sinensis

Recently, catechins have been recognized to be efficient antioxidant by scavenging reactive oxygen species (ROS), and this capacity is important insofar as ROS are involved in oxidative diseases [17]. Fig. 4 shows the ESI-MS positive mass spectrum of decaffeinated green tea extract dissolved in 50% methanol and 1% acetic acid in water.

The most abundant ion m/z 481 is due to sodium adduct of epigallocatechin-gallate (EGCg). The ions m/z 465, 329 and 291 are present at lower abundance, and correspond to the sodium adducts ($[M+Na]^+$) of epicatechin-gallate (ECg), epigallocatechin (EGC) and epicatechin/catechin (EC/C), respectively. Adding to the extract the cations potassium and cesium, it was possible to observe the corresponding shift of m/z values (see Table 1), thereby confirming the previous identification. ESI-MS data are in good agreement with those obtained by HPLC analysis [18].

3.5. Propolis

Propolis is a resinous hive product collected by bees from buds of different plants. Propolis contain many polyphenolic compounds including

flavonoids and cinnamic acid derivatives, that are thought to exert several biological activities [19].

Defatted samples of propolis (EPID) were analyzed by ESI-MS and the best results were obtained in negative mode. Fig. 5 shows a typical mass spectrum. The main ion (m/z 253) is due to chrysin, a flavone aglycone. The ions m/z 269 and 271 correspond to apigenin/galangin and naringenin, respectively. Pinocembrin is also present (m/z 255), but its abundance is lower.

3.6. Ginkgo biloba

Standardized extracts of *G. biloba* leaves are used in Europe for the treatment of peripheral and cerebral circulation disorders [20]. These ex-

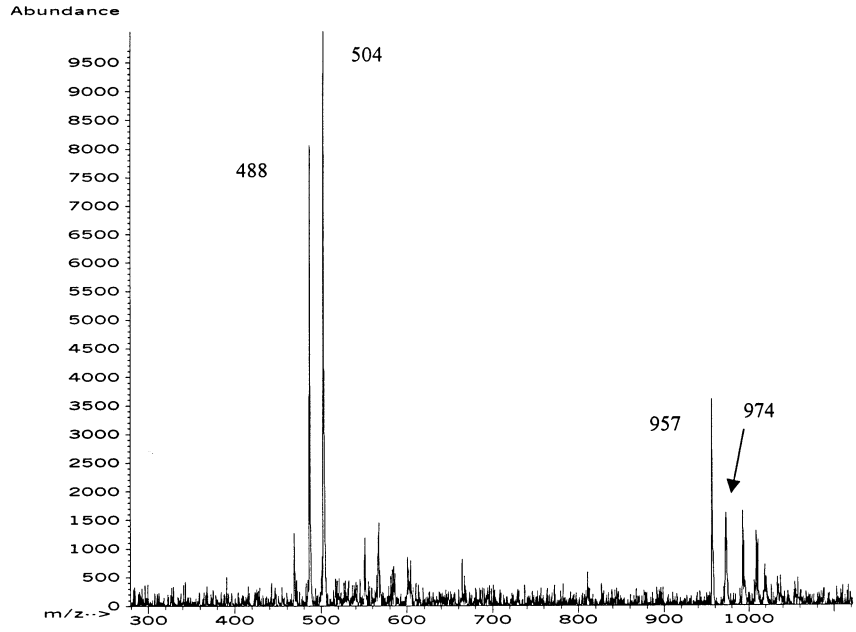

Fig. 3. Typical negative mass spectrum of C*entella asiatica* extract.

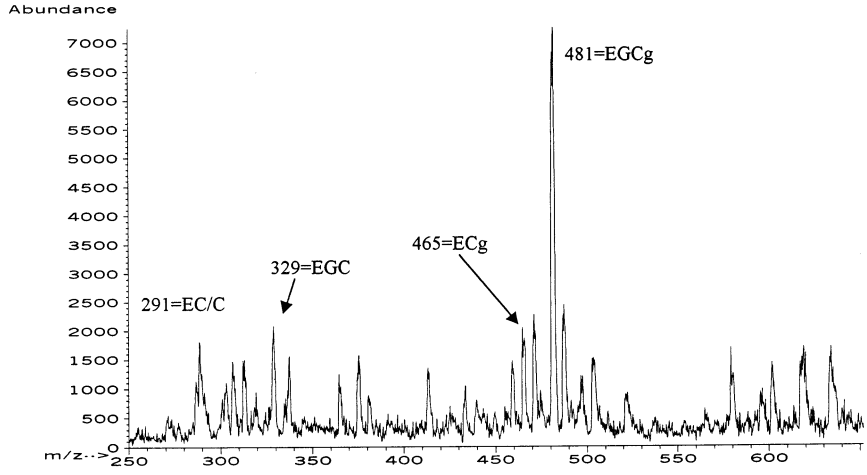

Fig. 4. Typical positive mass spectrum of green tea extract.

Table 1
Ions observed in green tea extract after adding different cations (sodium, potassium and cesium)

Compound	Abbreviation	MW	$[M+Na]^+$	$[M+K]^+$	$[M+Cs]^+$
Epigallocatechingallate	EGCg	458	481	496	591
Epicatechingallate	ECg	442	465	481	575
Epicatechin/catechin	EC/C	290	313	329	423
Epigallocatechin	EGC	306	329	345	439

tracts contain ginkgoflavonol-glycosides and terpenoids (ginkgolides and bilobalide).

The finger-print of *G. Biloba* extracts obtained by direct infusion in ESI-MS is shown in Fig. 6. Ions m/z 431 ([Ginkgolide A + $Na]^+$), 447 ([Ginkgolide B + $Na]^+$/[Ginkgolide J + Na^+]) and 463 ([Ginkgolide C + $Na]^+$), are clearly evidenced. The other ions (m/z 617–779) refer to different flavonol-glycosides. In particular, the ions m/z 617, 633 and 647 are due to the sodium adducts of kaempferol-3-O-rutinoside, quercetin-3-O-rutinoside and isorhamnetin-3-O-rutinoside, respectively, whereas the ions m/z 763, 779 and 793 correspond to the sodium adducts of -3-O-[rhamnosyl-(1→2)-rhamnosyl-(1→6)-glucoside] derivatives of kaempferol, quercetin and isorhamnetin, respectively.

To focus that this approach allows to detect simultaneously both classes of compounds (flavonoids and terpenes) present in *G. biloba* extracts.

3.7. Glycine max (soybean)

Soybean contains high concentrations of isoflavones either as highly-polar glycoside conjugates (daidzin and genistin), or in the free form, e.g. daidzein and genistein. These compounds exert a weak estrogenic effect on the hypothalamic-pituitary-gonadal axis, and potentially may be beneficial against the risk of hormone-dependent cancers [21].

Fig. 7 shows the positive mass spectrum of soybean extract dissolved in methanol 50% and 1% formic acid. The main ion (m/z 255) is due to the aglycone daidzein, whereas the glycoside daidzin (m/z 417) and genistein (m/z 271) are present at lower abundances.

3.8. Cynara scolymus (artichoke)

Artichoke is characterized by phenolic acid constituents, including caffeic acid, mono- and di-caffeoyl quinic acids (e.g. cynarin) and chlorogenic acid. These compounds are thought to be responsible for the choleretic, hypocholesterolemic

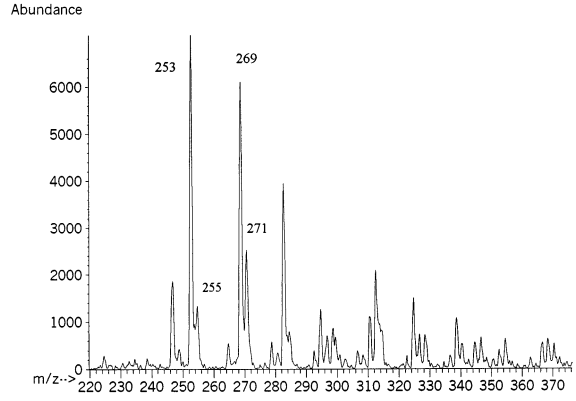

Fig. 5. Typical negative mass spectrum of propolis.

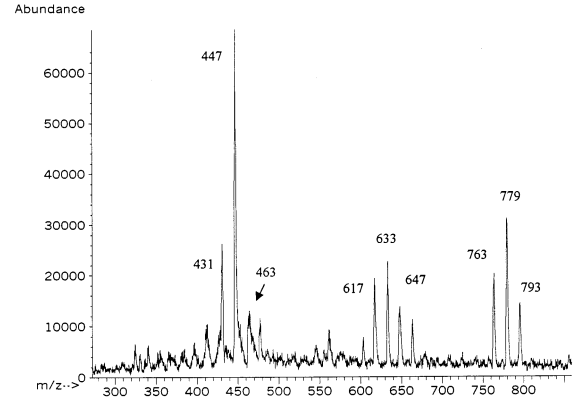

Fig. 6. Typical positive mass spectrum of *Ginkgo biloba* extract.

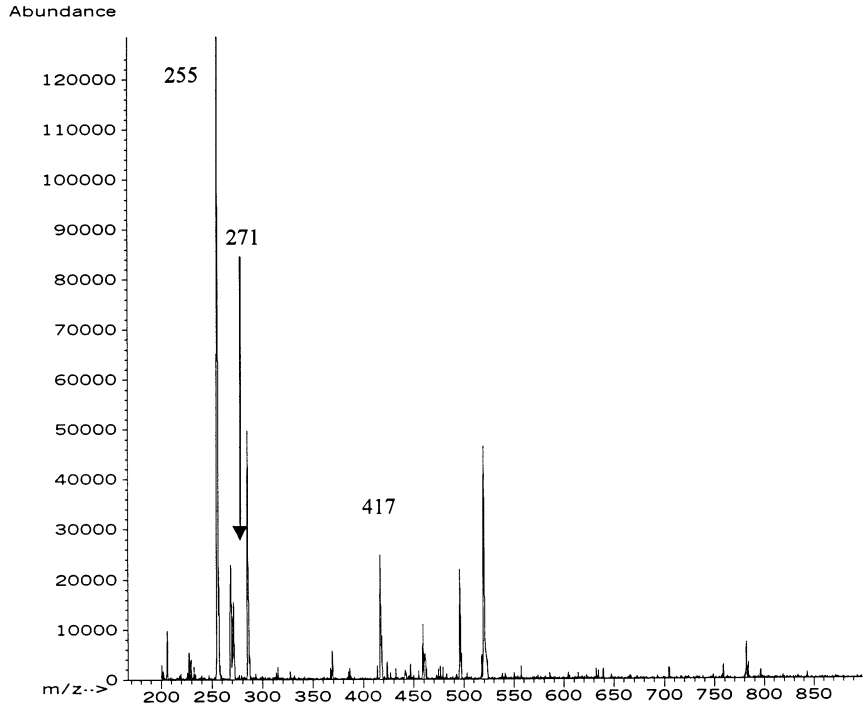

Fig. 7. Typical positive mass spectrum of soybean extract.

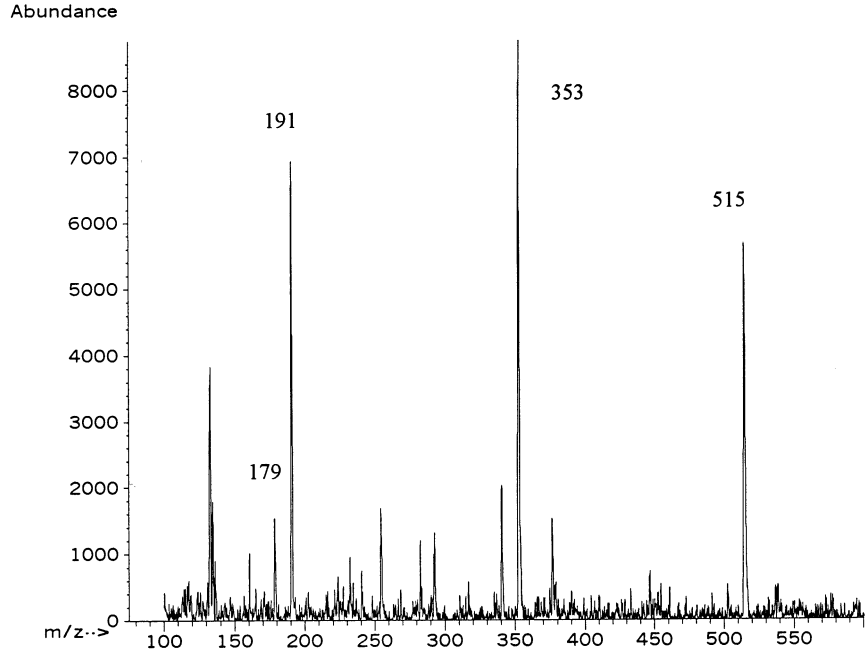

Fig. 8. Typical negative mass spectrum of artichoke extract.

and hepatoprotective properties traditionally attributed to artichoke [22]. Analyzing artichoke extract in negative mode it was possible to obtain a finger-print of phenolic acids present (Fig. 8). The main ions m/z 191, 353 and 515 correspond to quinic, chlorogenic and cynarin, respectively. Caffeic acid (m/z 179) is also present.

4. Conclusions

ESI-MS approach by direct infusion allows to detect the bioactive compounds in complex matrices of plant extracts. This method is specific, sensitive, rapid and does not require pre-purification steps.

The described approach provides the finger-print of herbal extracts, since it permits to detect simultaneously different constituents present in the sample, and can be considered a valuable tool for the qualitative control of herbal extracts.

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References

[1] P.G. Pietta, P.L. Mauri, J. Chromatogr. 549 (1991) 367–373.
[2] P.G. Pietta, P.L. Mauri, C. Gardana, J. High Resoln. Chromatogr. 17 (1994) 616–618.
[3] P.L. Mauri, G. Catalano, C. Gardana, P.G. Pietta, Electrophoresis 17 (1996) 367–371.
[4] I. Merfort, P.G. Pietta, P.L. Mauri, L. Zini, G. Catalano, G. Willuhn, Phytochem. Anal. 8 (1997) 5–8.
[5] P.G. Pietta, P.L. Mauri, R. Bauer, Planta Med. 64 (1998) 649–652.
[6] B. Stenke, B. Müller, H. Wagner, Planta Med. 59 (1993) 155–160.
[7] F. Camponovo, J.F. Wolfender, M.P. Maillard, O. Potterat, K. Hostettmann, Phytochem. Anal. 6 (1995) 141–148.
[8] M. Brolis, B. Gabetta, N. Fuzzati, R. Pace, F. Panzeri, F. Peterlongo, J. Chromatogr. A 825 (1998) 9–16.
[9] P.G. Pietta, R. Maffei Ficino, M. Carini, P.L. Mauri, J. Chromatogr. 661 (1994) 121–126.
[10] E. Schröder, I. Merfort, Biol. Mass Spectrom. 4 (1991) 11–15.
[11] P.L. Mauri, L. Iemoli, C. Gardana, P. Riso, P. Simonetti, M. Porrini, P.G. Pietta, Rapid Commun. Mass Spectrom. 13 (1999) 924–931.
[12] M. Grieve, A Modern Herbal, vol. I, Hafner, New York, 1967, pp. 99–100.
[13] P. Morazzoni, E. Bombardelli, Fitoterapia 67/1 (1996) 3–29.
[14] A. Piovan, R. Filippini, D. Favretto, Rapid. Commun. Mass Spectrom. 12 (1998) 361–368.
[15] K. Takagi, H. Saita, H. Nabata, Jpn. J. Pharmacol. 22 (1972) 245–249.
[16] C. Newall, C.A. Anderson, J.D. Phillpson, Herbal Medicines-A Guide for Health-Care Professionals, Pharmaceutical Press, London, 1996, pp. 170–172.
[17] J. Vinson, A. Dabbagh, M. Serry, J. Jang, J. Agr. Food Chem. 43 (1995) 2800–2802.
[18] P.G. Pietta, P. Simonetti, C. Gardana, A. Brusamolino, P. Morazzoni, E. Bombardelli, BioFactors 8 (1998) 111–118.
[19] T. Namba, Phytomedicine 3 (1996) 203–209.
[20] T.A. Van Beek, E. Bombardelli, P. Morazzoni, F. Peterlongo, Fitoterapia 3 (1998) 195–244.
[21] A. Cassidy, Proc. Nutr. Soc. 55 (1996) 399–417.
[22] C. Newall, C.A. Anderson, J.D. Phillpson, Herbal Medicines-A Guide for Health-Care Professionals, Pharmaceutical Press, London, 1996, pp. 36–37.