Available online: www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic 1842-4309

Not Bot Horti Agrobo, 2015, 43(2):349-354. DOI:10.15835/nbha43210028

AcademicPres

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Extraction and Characterization of Phenolic Compounds from Rose Hip (*Rosa canina* L.) Using Liquid Chromatography Coupled with Electrospray Ionization - Mass Spectrometry

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Abstract

Wild berry are a rich of natural compounds which provide them high antioxidant potential. The compounds which provide them these proprieties are known to be vitamins, flavonoids, anthocyanins and phenolic acids. The aim of this study was to extract, identify and characterize bioactive compounds from rose hip (*Rosa canina* L.) currently found in Romania. A qualitative high-performance liquid chromatography coupled with electrospray ionization mass spectrometric (ESI-MS) detection in positive ion mode has been used to identify phenolic compounds from rose hip crude extract. The chromatograms revealed the presence of a large number of compounds (19), identified and grouped as phenolic acids and flavones/ols, flavan-3-ols and also anthocyanins. Based on obtained results these berries can be highly recommended as part of our diet. Also this finding represents a contribution to the chemical characterization of phenolic profile of rose hip.

Keywords: anthocyanins, flavonoids, HPLC, phenolic acids, rose hip

Introduction

The genus *Rosa* L. (*Rosaceae*) covers more than 100 wild species and thousands of cultivars, which are widespread and grow in the north part of Europe, Asia, Middle East and North America (Rehder, 1940; Gu *et al.*, 2003). *Rosa canina* L. (rose hip) is an important berry for food industry due to its rich chemical composition. The small berries are an important source of colour, flavours and bioactive compounds with potential health benefits (Hvattum, 2002; Wu *et. al.*, 2005; He *et al.*, 2010; Jodee *et al.*, 2011; Rugină *et al.*, 2012; Tayefi-Nasrabadi *et al.*, 2012; Widen *et al.*, 2012; Dulf *et al.*, 2013; Yang *et al.*, 2015). Rose hip contains compounds with antioxidant potential such as ascorbate, ß-carotene, glutathione, a-tocopherol, anthocyanins and other phenolics (Ercisli, 2007; Nojavan *et al.*, 2008; Tozzi *et al.*, 2008; Tumbas *et al.*, 2012; Czyzowska *et al.*, 2015). So far, fruits of *Rosa canina* L. *(Rosaceae),* rose hips, are known for their high content of vitamin C and for their great flavour. *Rosa canina* L. grows naturally in Romania and, as reported previously, fruit and flavoured teas are very popular in many European countries (Pękal *et al.*, 2011). Rose hip can be consumed in both raw and dried state, being used in the herbal tea for flavour and a natural dose of vitamin C. The chemical composition of rose hip has been very rarely evaluated in comparing with other berries containing similar compounds. The polyphenols contained by rose hip are a large group of secondary metabolites. They are widely distributed in vegetables and berries ranging from simple molecules, such as phenolic acids, to complex molecules with numerous phenolic groups, e.g. acylated flavonoid glycosides, proanthocyanidins or tannins. Polyphenols occur primarily in conjugated form, linked to sugars moieties, and to other compounds, such as carboxylic and organic acids, amines, lipids and even to other polyphenols (Bravo, 1998; Guimarães *et al.*, 2013).

Rose hip consumption has been associated with preventive and therapeutic proprieties against a wide range of degenerative diseases, including the inflammatory arthritis disorder, rheumatism, gastrointestinal disorders or cancer (Larsen *et al.*, 2003; Rein *et al.*, 2004; Christensen *et al.*, 2008; Fujii *et al.*, 2009; Andersson *et al.*, 2012). In a recent study Widen *et al.* (2012) has evaluated erythrocyte antioxidant protection of rose hips. They have investigated the degree of amelioration of oxidative damage in an erythrocyte in vitro bioassay by comparing the effects of a reducing agent on erythrocytes alone with the effect on erythrocytes pretreated with berry extracts. The obtained results revealed that the maximum protection against oxidative stress, 59.44%, was achieved when incubating the cells with the first eluted meta-phosphoric extract. Removal of ascorbic acid from this extract increased the protection against oxidative stress to 67.9%. These results clearly indicate that rose hips contain a

Received: 08 July 2015. Received in revised form: 13 Sept 2015. Accepted: 15 Sept 2015. Published online: 10 Dec 2015.

promising level of clinically relevant antioxidant protection (Widen *et al.,* 2012). Another published study reports that the rose hip fractions rich in flavonoid inhibit cell proliferation in HeLa, MCF7 and HT-29 cell lines (Tumbas *et al.,* 2012). The potential health benefits of rose hips are due to the polyphenolic compounds found in these berries that are mainly flavonoid, phenolic acids and anthocyanins.

Since these classes of compounds are normally found as complex molecules in natural sources, several techniques have been developed for their separation and identification: mass spectrometry (MS) is the most used with several techniques, such as electrospray ionization mass spectrometry (ESI-MS), liquid chromatography coupled with mass spectrometry (LC/MS), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) coupled with mass spectrometric (MS/MS), high performance thin layer chromatography (HPTLC) and also nuclear magnetic resonance NMR (Nawwar *et al*., 1994; Wang *et al*., 1999; Reed *et al*., 2005; Mandal *et al*., 2008; Ferrari *et al*., 2011; Savage *et al*., 2011; Hingse *et al*., 2014; Ali *et al*., 2015; Jin *et al*., 2015). Being one of the most common fruit used in our country as herbal tea, the proposal of this work was to characterize their chemical profile. Therefore, the objective of the present study was to identify individual phenolic compounds in rosehip using the HPLC-ESI-MS.

Materials and Methods

Plant material

Fully ripened rose hips (*Rosa canina* L.) from spontaneous flora of Transylvania (Cluj, Feleac) were harvested during the months of September and October 2011. Samples were randomly collected from several plants and the form and variety of biotype to be analysed was taxonomically classified after a preliminary botanical identification. The identified sample was *Rosa canina* var. *lutetiana* f. *flexibilis*. Rose hips without calyxes were washed with water and kept in a freezer at -20 °C prior to the analysis.

Chemicals

All solvents, reagents and standards used to perform the experiments were of analytical grade. Methanol, formic acid (purity 98-100%) and hydrochloric acid 32% were provided by Sigma-Aldrich (Darmstadt, Germany). Anthocyanins standards, cyanidin-3-O-glucoside chloride, cyanidin-3-O-galactoside (purity 90%), cyanidin-3-arabinoside (purity 97%), cyanidin-3-O-glucoside (purity 95%) and cyanidin (purity 95%) were purchased from Polyphenols (Norway) while phenolic acids as gallic acid, protocatecuic acid, chlorogenic acid, catechin, caffeic acid, vanilic acid, rutin, ellagic acid, p-coumaric acid, ferulic acid, myricetin, tilirosid, quercetin and kaempferol were all purchased from Sigma-Aldrich, Darmstadt, Germany.

Extraction of polyphenolic compounds from rose hip

The method used to obtain crude extract rich in phenolic compounds was done according to previous studies with minor modifications (Cuevas-Rodriguez *et al*., 2010; Rugină *et al*., 2012). Berries samples, 1 g each, were grounded using an ultraturax (Miccra D-9 KT Digitronic, Germany) and weighed, followed by addition of 10 ml of acidified methanol (0.3% HCl (v/v)). The extraction process was repeated until the samples were colourless. The acidic condition was created in order to prevent anthocyanins from degradation. The obtained extracts were filtered through multiple layers of cotton and concentrated at 35 °C under reduced pressure (Rotavapor R-124, Buchi, Switzerland). Thereafter, it was dissolved in a known amount of acidified water, filtered through 0.45 μm Millipore filter and analyzed by liquid chromatography.

HPLC-DAD/-ESI-MS identification of anthocyanins, flavonol glycosides and phenolic acids

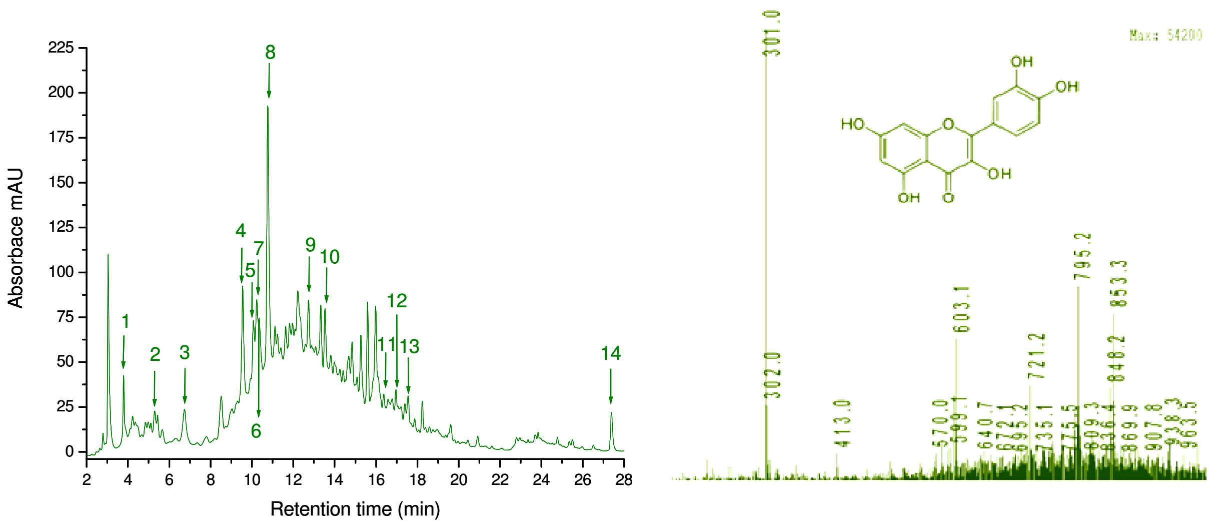
Samples were analyzed on an Agilent 1200 system equipped with a binary pump delivery system LC-20 AT (Prominence), a degasser DGU-20 A3 (Prominence), a diode array SPD-M20 A and an UV–VIS detector (DAD). Volumes of 10 μl were injected on an Eclipse XDB-C18 (5μm, 150x4.6 mm) column. The mobile phase consisted in: solvent A - bidistilled water and 0.1% formic, B – acetonitrile. The gradient elution system started with 10% B for 9 min. The percent of B increased to 12% at 17 min and continued up to 25% B at 30 min, between minute 30 and 50 the percentage of B being 90%. DAD recorded full spectra. In-line MS data were recorded by directing the LC flow to a Quadrupole 6110 mass spectrometer (Agilent Technologies, Chelmsford, MA) equipped with an ESI probe. Flow rate was 0.5 ml/min and column temperature was maintained at 25 °C. The chromatograms were monitored at 280, 340 and 520 nm, respectively. The compounds identification and peak assignments were done based on their retention times, UV-VIS spectra and also compared with standards and published data. As a confirmation the samples were analyzed by HPLC-ESI-MS. The measurements were performed in the positive mode with an ion spray voltage of 3000 V, and a capillary temperature of 350 °C. Data were collected in full scan mode within the range 280 to 1000 m/z. Identification of anthocyanins, flavonol glycosides and phenolic acids was carried out based on molecular mass determination, masses and occurrence of fragments, elution order and literature data reported previously (Koponen *et al*., 2007; Adam *et al*., 2013; Guimarães *et al*., 2013).

Results and Discussion

Rose hip (*Rosa canina* L.) varieties were not extensively studied, due to this fact the present study relieved that they are a rich source of anthocyanins, flavonol glycosides and phenolic acids. The identification of phenolic compounds present in rose hip crude extract was performed by comparing their UV-Vis spectra with published data and available standards, as well, trough HPLC-DAD-ESI/MS. A total of 19 different types of phenolic compounds were identified in crude extract. The main classes of phenolic compounds identified were grouped in phenolic acids and flavones/ols, flavan-3-ols and also anthocyanins. HPLC profile of polyphenols was recorded at three wavelengths: 280 nm for phenolic acids, 340 nm for flavan-3-ols and 520 nm for anthocyanins. The obtained chromatograms with each identified peak are showed and discussed below.

Phenolic acids and flavones/ols

In crude extract of rose hip, hydroxycinnamic acids were the major class of phenolic acids which is in agreement with literature (Hvattum, 2002; Guimarães *et al.,* 2013). Peak assignments of the different identified compounds are presented



A B

Fig. 1. HPLC chromatogram of rose hip crude extract at 280 nm (A) used for recording phenolic acids and flavones/ols and mass spectrum under peak 14 (B) analysed with LC/MS using positive ESI

Table 1. HPLC-MS tentative identification of phenolic acids in rose hip crude extract

Peak no	Rt (min)	λmax [nm]	Molecular ion [M-H]+ (m/z)	Tentative identification	Identification type
1	3.7	352	435/301	Quercetin-pentoside	DAD/MS
2	5.2	289	451/(289, 153, 137)	Eriodictyol hexoside	DAD/MS
3	6.7	355	479/303	Quercetin hexuronide	DAD/MS
4	9.5	358	463/301	Quercetin 3-O-glucoside	Standard DAD/MS
5	10.0	354	479/301	Quercetin glucuronide	DAD/MS
6	10.2	355	479/301	Quercetin hexuronide	DAD/MS
7	10.4	298	347, 185, 163	Methyl gallate-hexoxid	DAD/MS
8	10.7	304	229	Resveratrol	DAD/MS
9	12.7	325	355, 180	3-Caffeoylquinic acid	DAD/MS
10	13.5	280	595, 287	Kaempferol rhamnosyl-hexoside	DAD/MS
11	16.3	250 340	566	Apigenin (+galactoside-arabinoside)	DAD/MS
12	16.9	250 363	340	Coumaroylic acid (isomer 3-p)	DAD/MS
13	17.2	348	701	Tilirosid	Standard DAD/MS
14	27.7	360	302	Quercetin	Standard DAD/MS

in Table 1. The analysed crude extract was characterized by the presence of a large number of phenolic acids and flavones/ols, more specifically 14 individual compounds (Fig. 1).

Quercetin derivates which have λmax around 355 and an MS fragment at m/z 301 were mainly identified in rose hip crude extract (peak 1, 3, 4, 5, 6, 14). Structure, fragmentation, full ESI-MS and spectra of quercetin is showed in Fig. 1.

Detected quercetin derivates were assigned to quercetin pentoside ([M-H] at m/z 435); peaks 3-6 ([M-H] at m/z 479) were tentatively identified as quercetin hexuronide, quercetin 3-O-glucoside, quercetin glucuronide and quercetin hexuronide. This identification of quercetin glycosides was done based on process of releasing a 301 (m/z) ion fragment in the MS/MS spectrum.

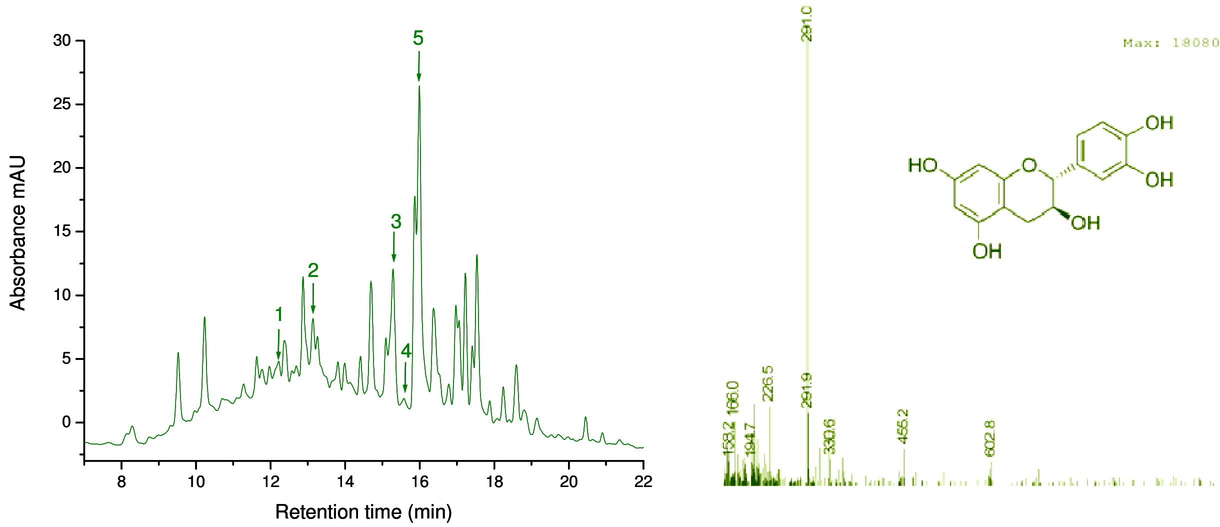
Other phenolic acids detected in rose hip crude extract were identified as 3-caffeoylquinic acid and coumaroylquinic acid (isomer 3-p) (peak 9, 12) based on the fragmentation patterns described previously by Clifford *et al.*, 2006. Peak 10 ([M-H] at m/z 595) was identified as kaempferol rhamnosyl-hexoside.

The obtained results are in agreement to available literature (Jin *et al.*, 2015). However, to the best of our knowledge, this is the first report that relieved the presence of resveratrol in the rose hip crude extract (Fig. 1 at 280 nm).

Flavan-3-ols

Catechin (flavan-3-ols) is the most frequent form of flavanols that occurs in plants (Vagiri *et al.*, 2012). The identified flavan-3-ols in rose hip crude extract are catechin and catechin hexoside, their galloyl derivates, according to their comparison with standards, published data and also confirmed by ESI-MS analysis.

The presence of catechin in rose hip has been previously reported (Hvattum, 2002; Guimarães *et al.*, 2013). We identified peaks 1 as catechin with ([M-H] at m/z 290). Their derivates were identified as catechin methyl gallate with ([M-H] at m/z 291) and catechin hexoside with ([M-H] at m/z 475). The HPLC-ESI-MS fingerprint from the rose hip crude extract is shown in Fig. 2. Peak assignments of the different identified compounds and their MS spectra are shown in Table 2.

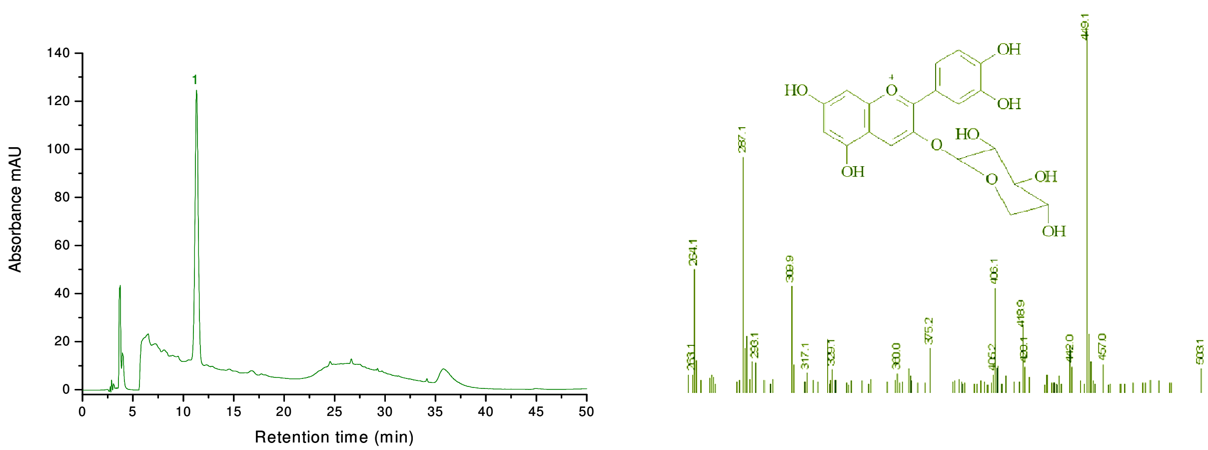


A B

Fig. 2. HPLC chromatogram of rose hip crude extract at 340 nm (A) used for recording flavan-3-ols and mass spectrum under peak 1 (B) analysed with LC/MS using positive ESI

Table 2. HPLC-MS tentative identification of flavan-3-ols in rose hip crude extract

Peak no	Rt (min)	λmax [nm]	Molecular ion [M-H]+ (m/z)	Tentative identification	Identification type
1	12.2	280	290	Catechin	Standard DAD/MS
2	13.7	278	185	Dimmer Methyl gallate	DAD/MS
3	15.2	280	475	Catechin methyl gallate	DAD/MS
4	15.5	280	291	Catechin hexoside	DAD/MS
5	15.9	280	436, 175	ND	



A B

Fig. 3. HPLC chromatogram of rose hip crude extract at 520 nm (A) used for recording anthocyanins and mass spectrum under peak 1 (B) analysed with LC/MS using positive ESI

Recent performed studies relieved that rose hip contains significant amounts of phenolic acids with antioxidant activity (Roman *et al.*, 2013), the major compounds being catechin, a proanthocyanidin monomer, a proanthocyanidin dimmer, rutin, quercetin galactoside, quercetin glucoside and cyanidin-glucoside (Widen *et al.*, 2012). The identified compounds in this study are in agreement with published data related to the phenolic compounds in rose hip (Hvattum, 2002; Widen *et al.*, 2012; Guimarães *et al.*, 2013).

Table 3. HPLC-MS tentative identification of anthocyanins in rose hip crude extract

Peak no	Rt (min)	λmax [nm]	Molecular ion [M-H]+ (m/z)	Tentative identification	Identification type
1	11.3	516	449, 287	Cyanidin-3-O- glucoside	Standard DAD/MS

Anthocyanins

The anthocyanins profile obtained for rose hip crude extract consists in only one compound (Fig. 3). The chemical characteristics regarding the identity and mass spectrum of the anthocyanin found in the analysed sample is presented in Table 3.

Conclusions

The present study provides for the first time information regarding the chemical composition of *Rosa canina* var. *lutetiana* f. *flexibilis*. In this study, 19 individual types of phenolic compounds were identified such as anthocyanins, flavonol glycosides and phenolic acids. Moreover, these results represent an important contribution to the chemical profile characterization of phenolic compounds using HPLC-ESI-MS and diode-array detection from rose hip crude extract. However, further studies are required in order to evaluate the percentage of each compound and their antioxidant potential.

Acknowledgments

This work was supported by the Romanian Ministry of Education and Scientific Research, CNCSIS – UEFISCDI, PN-II-RU-PD-2012-3 – 0055, grant nr. 47/30.04.2013.

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