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Anthocyanins profile of *Vaccinium myrtillus* alcoholic extracts revealed by electrospray ionization/mass spectrometry

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Abstract. Alcoholic extracts of blueberries, *Vaccinium myrtillus* (bilberry), were analyzed by electrospray ionization - mass spectrometry (ESI-MS), in order to identify the anthocyanins in these extracts. The anthocyanin aglycons, cyanidin, peonidin, petunidin and some glycosides such as petunidin 3-rutinoside, peonidin 6-acethyl-3-glucoside, malvidin 3-arabinoside, delphinidin 3-arabinoside, cyanidin 3-xyloside (or cyanidin 3-arabinoside) were confirmed to be present in *Vaccinium myrtillus* extracts.

Keywords: anthocyanins extract, *Vaccinium myrtillus*, ESI-MS.

1. Introduction

Anthocyanins are a group of natural pigments responsible for the red-blue colour of many fruits and vegetables, being the secondary metabolites produced by the plants. This class of compounds is interesting because they can be used in the food technology as natural colorants [1] and also have important implications in the human health. Numerous studies indicate the potential effect that this family of flavonoids may have in reducing the incidence of cardiovascular diseases, cancer, hyperlipidemias [2] and other chronic diseases through the intake of anthocyanin-rich foods [3].

Many type of fruits, like bilberry, blackberry, gooseberry, from berries fruits class, have a high content of flavonoids and polyphenols, compounds with recognized antioxidant activity. This property is due to the hydroxy groups, which can neutralize the oxygen radicals (responsible for initiating and propagating the oxidation reactions) resulting more stable compounds [4]. Main flavonoid subgroup in fruits (berries) are anthocyanins. Lowbush and highbush blueberries have an unusual content of significant amounts in the five aglycones (Fig. 1): delphinidin, cyanidin, petunidin, peonidin and malvidin [5].

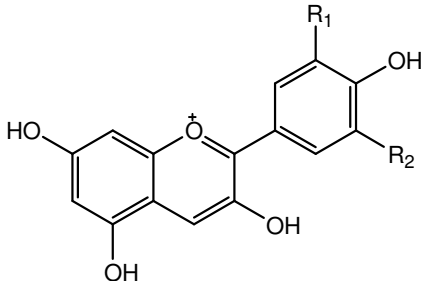

Fig. 1. Structural formula of anthocyanidins.
cyanidin, $R_1 = OH$, $R_2 = H$;
delphinidin, $R_1 = R_2 = OH$;
peonidin, $R_1 = OCH_3$, $R_2 = H$;
petunidin, $R_1 = OCH_3$, $R_2 = OH$;
malvidin, $R_1 = R_2 = OCH_3$.

Bilberry is one of a low bush wild blueberry found in Northern Europe natively. Its extract has been recognized as a medicine in Italy in ophthalmology [6].

ESI-MS is a powerful tool for the analysis of anthocyanins [7, 8], allowing the simultaneous determination of all anthocyanins in plant extracts. The aim of this study was to identify the anthocyanins in the *Vaccinium myrtillus* alcoholic extracts using electrospray ionization/mass spectrometry (ESI/MS) analysis.

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2. Experimental

Commercially *Vaccinium myrtillus* dried fruits, previously crushed, were extracted successively for 5 days with 96% ethanol (1:10 (w/v)). The mixture was centrifuged at 2000 rpm for 6 min and the supernatant was collected, filtered off and stored in a freezer at 5 ºC for analysing it the next day. Because polyphenols are extremely sensitive to light [9], all procedures were conducted under dim light.

For MS investigation, the samples were dissolved in pure methanol, and both (+) ESI MS^1 and tandem mass spectra (+) ESI MS^2 were acquired.

Solvents were purchased from Sigma-Aldrich (analytical grade).

Mass spectra were recorded in methanol on a high capacity ion trap, HCT Ultra PTM instrument (Bruker, Daltonics, Bremen), interfaced to a PC running the Compass™ 1.2. integrated software package, which includes the Hystar™ 3.2.37 module for instrument controlling and spectrum acquisition, Esquire Control™ 6.1.512 and Data Analysis™ 3.4.179 modules for storing the ion chromatograms and processing the MS data.

3. Results and discussions

MS^1 and mass calculation revealed in bilberry extract the presence of the molecular ions corresponding to the three anthocyanin aglycons, cyanidin (m/z=287.2), peonidin (m/z=301.3) and petunidin (m/z=317.3) (Fig. 2).

Anthocyanins found in bilberry consisted on delphinidin, cyanidin, petunidin, peonidin, and malvidin attached with rutinose, glucose, arabinose or xylose at the C-3 position.

In order to confirm the structure of these glycosides, fine and detailed structural analysis was performed by multistage mass spectrometry (MS^2).

In Fig. 3 it is presented the fragmentation of the molecular ion at m/z=419.4. It can be observed as fragment in this spectra the aglycon cyanidin (m/z=287.2), so that the ion at m/z=419.4 can be assigned to cyanidin 3-xyloside or cyanidin 3-arabinoside. The later is probably the one present in the bilberry extract according to Nakajima et. al. (2004), but the difference between the two glycosides can be made only by using a chromatography method (HPLC).

Figure 4 presents the MS^2 spectra of the ion at m/z=506.2, which can be assigned to peonidin 6-acethyl-3-glucoside. It can be observed in this fragmentation spectra the aglycon peonidin which appears at m/z=301.3.

The fragmentation of petunidin 3-rutinoside (m/z=625.5) is shown in figure 5. The fragment at m/z=317.1 corresponds to petunidin.

The molecular ions m/z=463.4 and m/z=611.3, isolated from bilberry extract MS^1, can be assigned to malvidin 3-arabinoside and delphinidin 3-arabinoside, respectively. The aglycons malvidin (m/z=331.4) and delphinidin (m/z=303.1) appear as fragments in MS^2 spectra (Fig. 6 and 7).

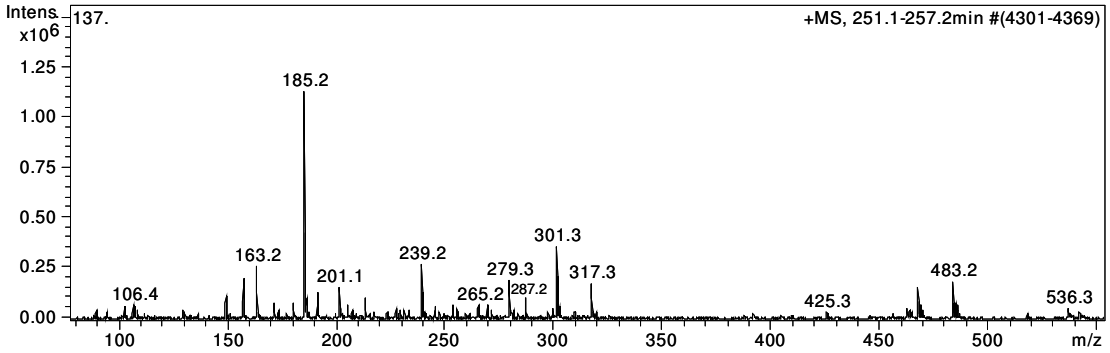

Fig. 2. (+) MS^1 analysis of bilberry extract

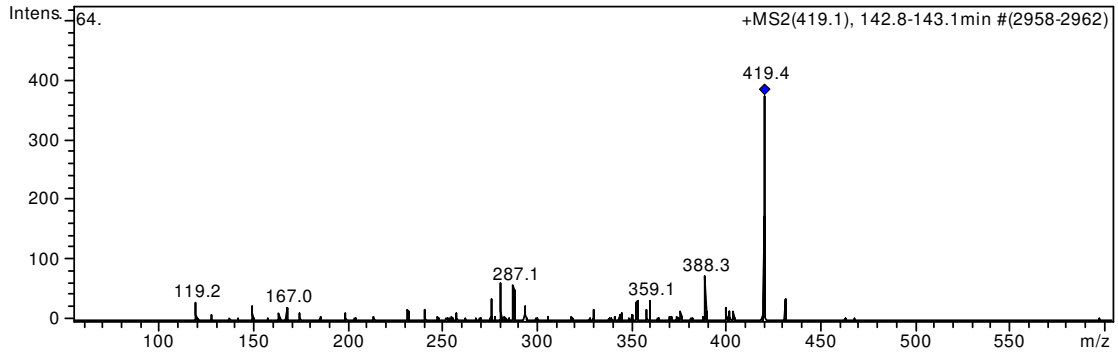

Fig. 3. MS^2 of the ion at m/z=419.4 detected in MS^1

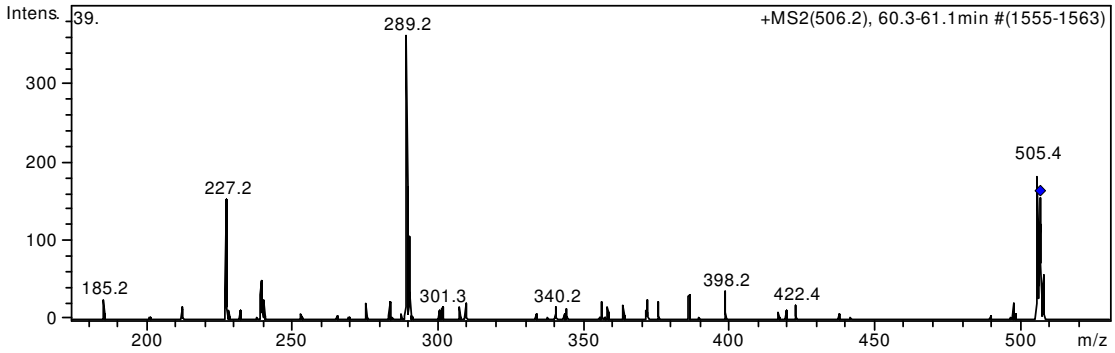

Fig. 4. MS^2 of the ion at m/z=506.2 detected in MS^1

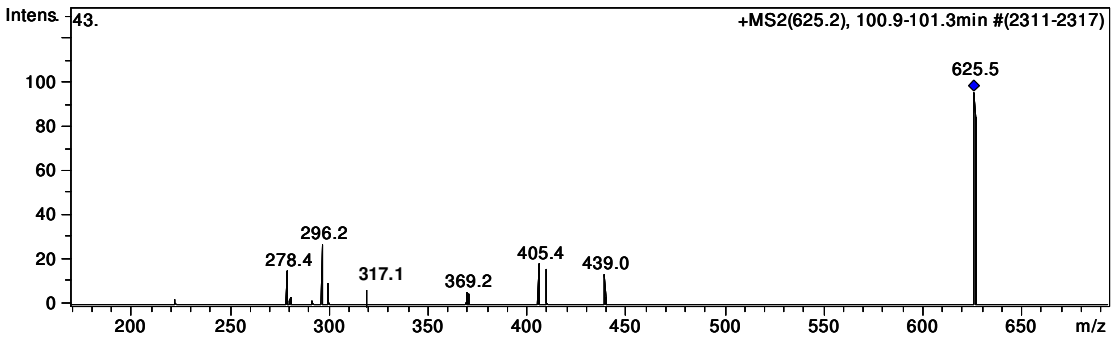

Fig. 5. MS^2 of the ion at m/z=625.5 detected in MS^1

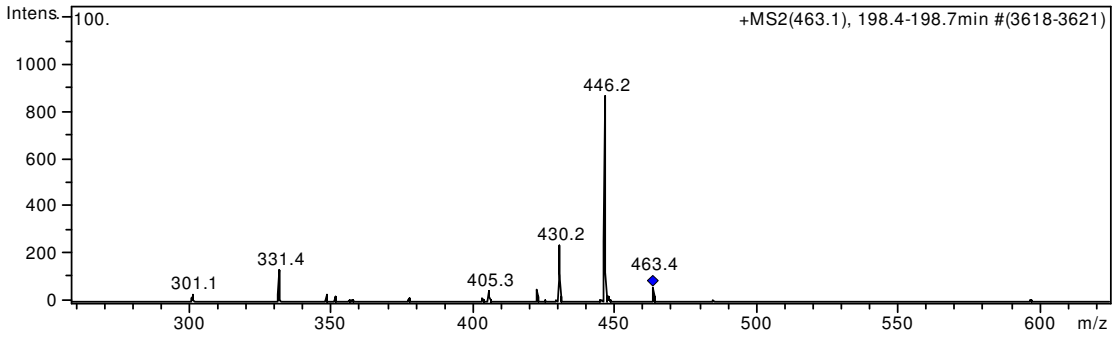

Fig. 6. MS^2 of the ion at m/z=463.4 detected in MS^1

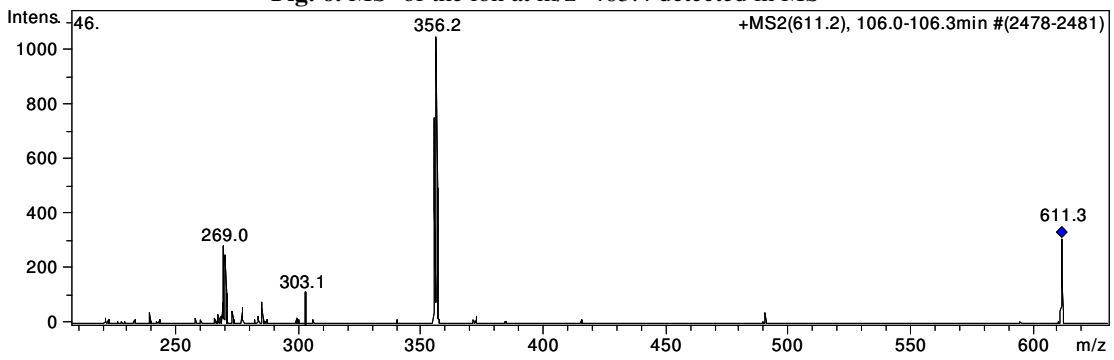

Fig. 7. MS^2 of the ion at m/z=611.3 detected in MS^1

4.Conclusions

Bilberry extract was confirmed to contain three anthocyanin aglycons, cyanidin, peonidin and petunidin.

Anthocyanins found in bilberry are delphinidin, cyanidin, petunidin, peonidin, and malvidin attached with rutinose, glucose, arabinose or xylose. Mass spectrometry is a very important tool in anthocyans identification, but in some cases, using chromatographic methods along the mass spectrometry represents a necessity, in order to assign the right structure of the investigated glycosides.

5. References

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