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SPECIFICATIONS OF THE CHEMICAL COMPOSITION AND THERAPEUTIC PROPERTIES OF *VACCINIUM VITIS-IDAEA* L. FRUITS

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Abstract: The aim of this study was to analyze the presence of arbutin in *Vaccinium vitis-idaea* fruits and to specify the importance of applying correct scientific names to avoid confusion. The therapeutic properties of natural products are closely related to their chemical composition. Whilst the leaves of *V. vitis-idaea* contain arbutin and other hydroquinone derivatives and can be used for their antiseptic properties, we did not identify any hydroquinone derivatives in the fruits of this species. The analyses were made using *Vitis idaeae folium* and *Vitis idaeae fructus* extracts, before and after acid hydrolysis, by TLC and HPLC-MS.

Keywords: *Vaccinium vitis-idaea*, arbutin, hydroquinone, HPLC-MS

Introduction

If the leaves of Cowberry, *Vaccinium vitis idaea* (*Vitis idaeae folium*), are well characterized chemically and pharmacologically, there is confusion as to the therapeutic use of the fruits, due to extrapolation of the properties of the leaves to the fruits. The leaves contain 4–6% arbutin, with antiseptic properties recommended to treat urinary tract infections (cystitis, pyelitis, nephritis), similar to the leaves of bearberry, *Arctostaphyllos uva-ursi*. Cowberry fruits are red because of anthocyanins located in the teguments. They also contain proanthocyanidins, phenolic acids derivatives (vacciniin = 6-benzoil-D-glucose), flavonoids, vitamins and mineral salts [1, 3, 5, 8].

The arbutin was identified only in the leaves, not the fruits. The fruits contain benzoic acid esters. Starting from the observation that cowberry fruits are not affected by mould development if preserved in water, Ujvari *et al*. identified a fungistatic activity related to the presence of the benzoic acid derivatives [10]: 150–180mg (Margheri, Micheloti), 55mg in fresh fruits and 310mg in dry fruits (Tamas *et al*.) [9]. The biosynthesis of the two aglycones is different: hydroquinone is formed by gentisic acid decarboxilation, benzoic acid by shortening the lateral chain of cinnamic acid β-oxidation [2].

The bacteriostatic effect of cowberry juice in the treatment of urinary infections is confirmed by historic use; this activity is attributed to the antiseptic properties of the benzoic acid derivatives (pH acid) and to the inhibition of bacterial adhesion to mucous membranes by the anthocyanidins [7].

Extracts obtained from the fruits of some *Vaccinium* species can be found on the Romanian market, in the composition of different phytotherapeutic products. To avoid confusion, it is important to note on these products the name of the species used.

For example, two products are available on the Romanian market in which the presence of cowberry extracts is noted in the composition. Thus, "syrup of cowberries" is described as a product with arbutin (incorrect statement) with bactericidal, diuretic and anti-inflammatory properties, indicated for the treatment of urinary infections and renal lythiase [13]. A second product, *Cran Clearance* (Secom), containing extract from the fruit of *Vaccinium macrocarpon*

(American cranberry, incorrectly named as cowberry), is indicated for its antioxidant and anti-inflammatory properties, as an adjuvant in the treatment of urinary tract infections and lythiase [12]. Dried fruits of *V. macrocarpon* are marketed in our country in the form of dehydrated fruits, imported and incorrectly labelled as "cowberries".

In this paper we propose to verify the presence of arbutin in indigenous cowberries and to point out the importance of knowledge of the scientific name to avoid any confusion. The chemical composition of natural products is very important for their therapeutic properties.

Materials and Methods

The leaves and the fruits of *Vaccinium vitis-idaea* were harvested from Baisoara (Cluj County) at 1350 m altitude in September 2011. The vegetal products were dried at room temperature and powdered. The fruit dry extract (1:2) was purchased from „Plantarom" Cluj-Napoca [11].

To analyze the arbutin, 1g of dry extract and then 0.5g of vegetal products (leaves and fruits) were extracted with 10mL of 50% methanol and 0.1g $CaCO_3$ and heat under a reflux condenser on a water bath for 15 minutes. After filtration, the volume of each sample was adjusted with the same solvent in a 10mL volumetric flask, and 0.1g of lead (II) acetate added to precipitate the tannins and flavonoids. After filtration, the A solution was obtained [4, 8].

The aglycones were analyzed after acid hydrolysis: 5 mL of A solution was treated with 5mL of 2N hydrochloric acid and the mixtures were heated at 80˚C on a water bath for 40 minutes. After cooling, the volume of each sample was adjusted with water in a 10mL volumetric flask and was then extracted twice with 5mL ethyl acetate in a separatory funnel. The ethyl acetate solutions were evaporated to dryness and the residue was dissolved in 1mL methanol (B solution) [4].

The arbutin was analyzed using TLC and HPLC, whilst the hydroquinone was analyzed by TLC.

TLC analysis of arbutin was made using the following chromatographic conditions [4]:

- stationary phase: silica gel G (Merck), 10x10cm, 0.2mm thickness
- mobile phase: ethyl acetate-methanol-water (100:16.5:13.5 v/v)
- standards: 0.1% methanolic solution of arbutin (Fluka AG), 1% methanolic solution of hydroquinone (Serva)
- migration distance: 7.5cm
- identification: 20% phosphotungstic acid in acetone followed by maintaining the plate in ammonia vapors

TLC analysis of hydroquinone was made using the solvent system toluene-ethyl acetate-formic acid (5:4:1) and the same chromatographic conditions as described [4].

HPLC-MS analysis [6]

Apparatus and chromatographic conditions: we used an Agilent 1100 HPLC Series (Agilent, USA) coupled with Agilent Ion Trap 1100 VL instrument. The HPLC apparatus was equipped with a HP 1100 Series auto-sampler, a Zorbax SB-C18 reversed-phase analytical column 100 mm x 3.0 mm i.d., 3.5 μm particle (Agilent technologies, USA), and we operated at 40^{o}C. The mobile phase was an isocratic elution using distilled water with 50 μM sodium acetate. The flow rate was 1 mL/min and the injection volume was 5 μL.

The detection was performed at 280nm, using a UV HP 1100 Series detector only for viewing the chromatographic profile, followed by single ion monitoring (SIM) mode using an ion trap mass spectrometer with electrospray positive ionization. For MS detection, m/z 295 ion was monitored, corresponding to the adduct between arbutin and sodium.

Nitrogen was used as the nebulizing and drying gas. The instrument was set to the following tune parameters: drying gas temperature 300°C, nebulizing gas pressure of 60psi, drying gas flow rate 12L/min, and capillary voltage +4000V.

The chromatographic data were processed using Chemstation and Data Analysis software from Agilent, USA.

Results and Discussion

ESI mass spectra of arbutin is presented in Figure 1 and the chromatogram of arbutin in MS detection (I) and UV detection (II) in Figure 2. The retention time for arbutin was 1.6min.

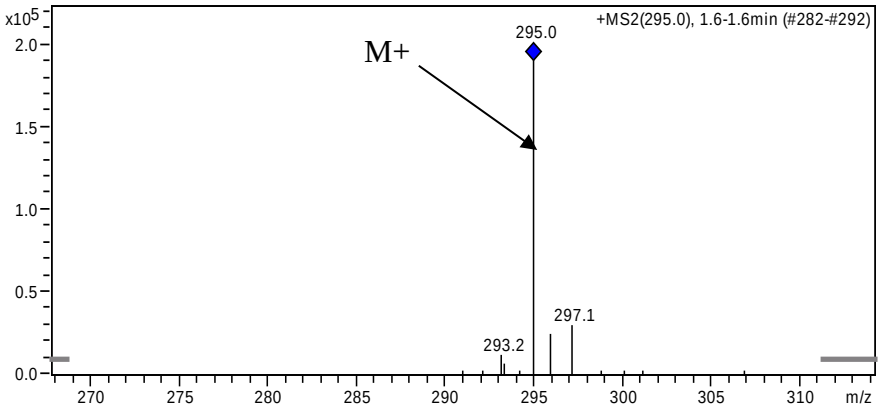

Fig. 1: ESI mass spectra of arbutin

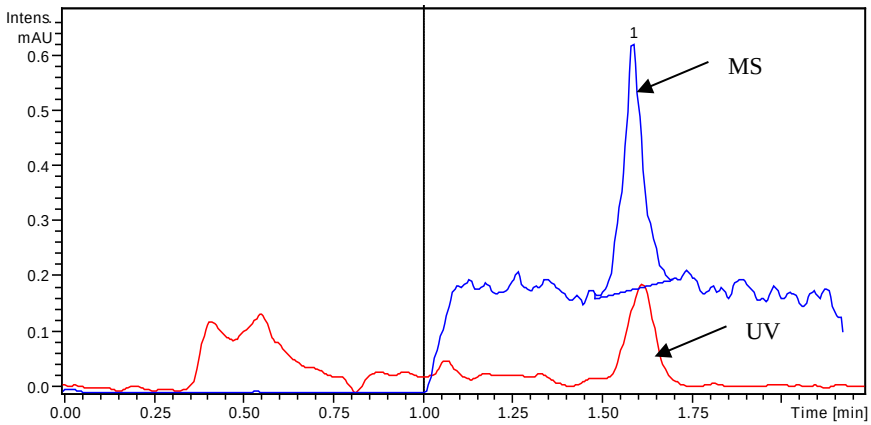

Fig. 2: Chromatogram of arbutin in MS detection (I) and UV detection (II)

Because arbutin is a polyhydroxylated compound, it can easily form adducts during ionization with metals such as sodium, potassium, lithium, etc. These metals are omnipresent and they can be found in very small concentrations (μM) in bidistilled water or reagents. The glass contains sodium and potassium silicates and may contaminate the bidistilled water with metallic ions. Usually, if a compound can form adducts with metals and these are more stable then the adduct of the substance with a proton, the last one cannot be marked out. The ion with m/z 295 corresponds to the adduct of arbutin with sodium (Fig.1).

The intensity of an adduct characteristic ion is proportional to the concentration of the substance analyzed, so it can be used for quantification. In this case, because of the high intensity

of the adduct with sodium, it was chosen as an ion on the basis of which the quantification of arbutin will be made.

50 μM sodium acetate solution was added to the mobile phase in order to be sure that the concentration of sodium will be adequate for the quantitative conversion of the analyte in the adduct. In these conditions, there will be enough sodium in the mobile phase to convert the full quantity of substance into the corresponding adduct.

The chromatograms of the samples analyzed are presented in Fig. 3.

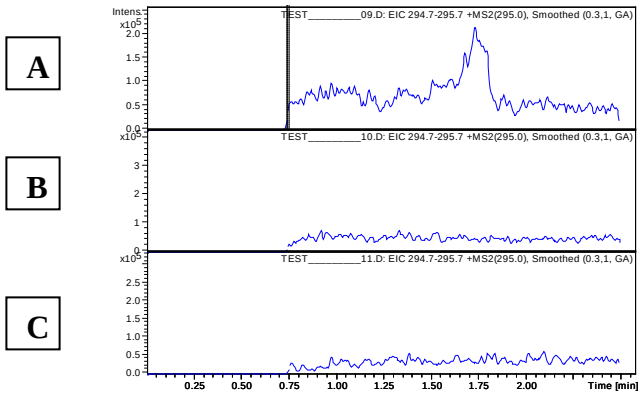

Fig. 3: The HPLC chromatograms of the samples: *Vitis idaeae folium* (A), *Vitis idaeae fructus* (B), *Vitis idaeae fructus* dry extract (C)

By TLC we identified arbutin only in the leaves of *Vaccinium vitis-idaea*, not in the fruits. We observed a greyish-blue spot at R_f=0.5 for arbutin and a blue spot at R_f=0.92 for hydroquinone. After acid hydrolysis of extracts of the leaves and fruits, hydroquinone was identified only in *Vitis idaeae folium* extract (R_f=0.55). Because after the hydrolysis we were unable to identify hydroquinone in the fruits of *V. vitis-idaea*, neither arbutin nor hydroquinone derivatives are present in *Vitis idaeae fructus*.

By HPLC-MS the presence of arbutin in leaves and its absence in fruits was confirmed.

These results show that the physiology and the biochemical metabolism of the two organs are different, even if they are from the same species: C_6 type compounds (hydroquinone, arbutin) are characteristic of the leaves, C_6–C_1 type compounds (benzoic acid, vacciniin) of the fruits.

Conclusion

The leaves of *Vaccinium vitis-idaea* can be used for their antiseptic properties because of their arbutin content, whereas the fruits do not contain this hydroquinone derivative. The antiseptic and antifungal properties of the fruits may be due to benzoic acid derivatives and anthocyanins, but not to arbutin.

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11. x x x Eridiarom, Prospect Plantarom Cluj Napoca.
12. x x x Secom Telverde Prospect Cran Clearance.
13. x x x Sirop de merişoare, Prospect PFA Mureşan Cr. Dealul Botii (Jud.Cluj).

PRECIZĂRI ASUPRA COMPOZIŢIEI CHIMICE ŞI PROPRIETĂŢILOR FRUCTELOR DE MERIŞOR (*VACCINIUM VITIS-IDAEA* L.)

(Rezumat)

Scopul acestui studiu a fost determinarea prezenţei arbutozidei în fructele de *Vaccinium vitis-idaea* şi de a preciza importanţa cunoaşterii nomenclaturii ştiinţifice pentru a evita unele confuzii. Proprietăţile terapeutice ale produselor naturale sunt determinate de compoziţia chimică. În timp ce frunzele de *V. vitis-idaea* conţin arbutozidă şi alţi derivaţi ai hidrochinonei şi sunt utilizate pentru proprietăţile antiseptice, în fructele acestei specii nu am identificat arbutozidă sau alţi compuşi derivaţi de hidrochinonă. Analizele au fost efectuate pe extracte de frunze şi fructe de merişor, înainte şi după hidroliza acidă, prin CSS şi HPLC.

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