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Dedicated to Professor Valer Fărcăşan at his 85th anniversary

ANTHOCYAN EXTRACTION FROM *VACCINIUM MYRTILLUS* FRUITS USING DIFFERENT EXTRACTION TECHNIQUES AND SPECTROPHOTOMETRIC AND TLC QUANTIFICATION

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ABSTRACT. The paper presents a study of anthocyan extraction from blubbery fruits - *Vaccinium mirtyllus fructus.* The used extraction methods were: Soxhlet, centrifugation, reflux, microwave and sonication. The total anthocyan content was determined spectrophotometrically and the delphinidine content was determined by TLC. The best results were obtained when the centrifugation was performed at room temperature. Also typical chromatograms for the extract are similar for all extraction techniques.

Key words: *Vaccinium myrtillus L. fructus, Soxhlet, extraction, Microwave extraction, Sonication, reflux, anthocyan.*

I. INTRODUCTION

The anthocyanidins (aglycones) are polyhydroxy and polymethoxy derivates of the 2-phenylbenzopyrylium cation. They are largely responsible for the scarlet through purple to blue colours of flowers, fruits, roots and leaves of higher plants, fruit juices, red wines, etc. They accumulate in the vacuoles of epidermal or subepidermal cells, but they may also be confined to the leaf mesophyll [1,2].

The bluberry (Vaccinium myrtillus) is a bushy shrub of the cowberry family, 15 to 40 cm high. Bluberries grow on flat terrain in shadowy pine and fir woods. A popular herb widely used in holistic medicine, the bluberry can be consumed fresh or dried, with or without sugar [3-5].

The bluberry is particularly rich in vitamin C, but also contains tanning agents, pectin's, organic acids, sugar, anthocyan dyes, carotenoids, and vitamin D. The leaves contain arbutin, a phenol glycoside. Fresh berries are a potent cure for gastric catarrh, while dried bluberries help relieve intestinal inflammation and diarrhea. When consumed in large quantities, fresh bluberries are a potent cure for gout, rheumatism and metabolic disorders [6,7].

The bioactive compound can be extracted using different techniques, like classical methods - maceration, reflux, Soxhlet [8-10] and modern methods - superfluid extraction (SFE), microwave assisted solvent extraction (MASE) and sonication [11-14].

The total anthocyan content, expressed in delphinidine, was spectrophotometrically measured at 536 nm, where delphinidine showed a maximum of absorbance (Merck Index). The TLC analysis was performed as described in [15] and quantity of extracted delphinidine was determined using calibration curve method.

II. EXPERIMENTAL

Different techniques were used to extract the bioactive compounds from *Vaccinium myrtillus* fruits. A sample of 1 g dried blubbery fruits was mixed with 2 g purified sand.

Extraction procedure

Soxhlet extraction was perform using 100 ml methanolic solution of HCl (5%) until a colourless extract was obtained. After solvent evaporation the residuum was solved with 10 ml methanol. The obtained sample was called A.

The room temperature extract was obtained by sample centrifugation (15 min. at 5000 rpm) with 10 ml methanolic solution of HCl. The extraction procedure was repeated two more times. The combined methanolic extracts were dried and dissolved in 10 ml methanol obtaining the sample B.

Reflux extraction was performed for 30 min. using 30 ml methanolic solution of HCl. The extract was dried and dissolved in 10 ml methanol. This was sample C.

Sonication was performed in a sonication bath (35 kHz) for 15 min. with 10 ml methanolic solution of HCl. The extraction was repeated two more time. The methanolic solutions were unified, dried and then dissolved in 10 ml methanol in order to obtain sample D.

Microwave extraction was performed with a home made apparatus ITIM Cluj-Napoca (microwave power systems 200-1200W, frequency 2.45GHz). The extraction procedure was applied for 2 min. with 30 ml methanolic solution of HCl, at an imput coefficient of 60%. After filtration the plant material was extracted under the same experimental conditions two more times. The combined methanolic extracts were dried up and then dissolved in 10 ml methanol obtaining the E extract.

TLC Analysis

Thin layer chromatography was performed on silica gel G F254 plates (Merck). The samples (extracts A-E) were applied on the plate using a Hamilton microsiringe as bands (20 µl). For quantitative delphinidine analysis, the applied volume was 10 µl/spot from methanolic solution with different concentrations: 1.2, 2.1, 2.7, 3.0, 3.9, 4.7, 5.5 and 6.1 mg/ml.

The plate was developed in a normal development chamber with n-butanol – acetic acid – water, 40:10:20 (v/v), as mobile phase. Densitometric evaluation of the chromatographic plate was performed in reflection mode at 536 nm using a Desaga CD 60 apparatus.

Spectrophotometric analysis

An aliquot of 0.05 ml methanolic extracts was diluted with methanol in a 10 ml volumetric flask and measured at 536 nm using methanol as reference solution. The calibration curve was performed using methanolic solutions with different compound concentration (0.005, 0.010, 0.015, 0.020 and 0.025 mg/ml).

III. RESULTS AND DISCUSSIONS

Chromatographic analysis

In figure 1 is presented the chromatogram of delphinidine (1) and of each plant extract.

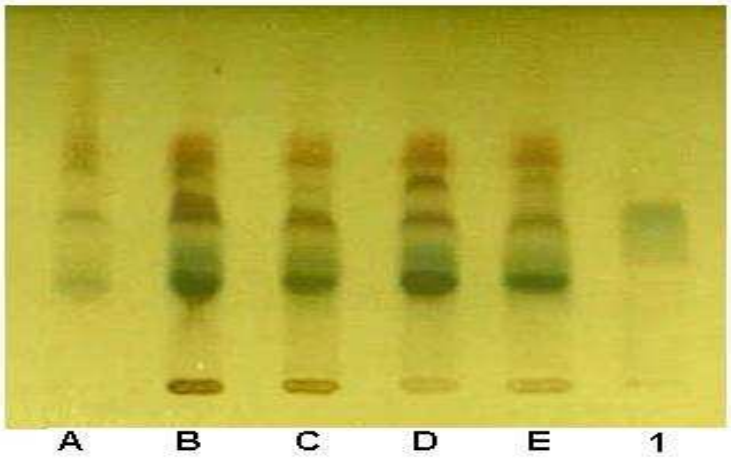

Figure 1. The typical chromatogram of delphinidine (1) and different blubbery fruit extracts (**A**, **B**, **C**, **D**, **E**)

The delphinidine spot was identified at R_f = 0.40. It can be observed that in every extract delphinidine is present in different quantities. Based on chromatograms presented in figure 1 we can observe that the fingerprints of the extracts are similar. These mean that there were no changes in the composition of extracts, no degradation processes of biocompound were occur. We can say that the extract composition does not depend up on the extraction techniques used.

The calibration curve and the coresponding parameters are presented in Figure 2.

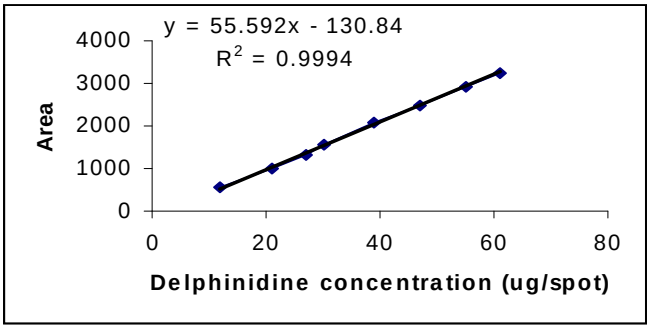

Figure 2. Calibration curve for delphinidine

In the Table 1 are presented the experimental data and the TLC determined delphinidine content.

Table 1.

Experimental data for determination of delphinidine content in the methanolic extracts.

Extract	Area	Delphinidine concentration (µg/spot)	Delphinidine content (mg/g)
A	1382	27.21	13.605
B	2878	54.12	27.06
C	2405	45.62	22.81
D	2420	45.89	22.945
E	2101	40.15	20.075

Spectrophotometric determination

The calibration curve and the parameters are presented in figure 3.

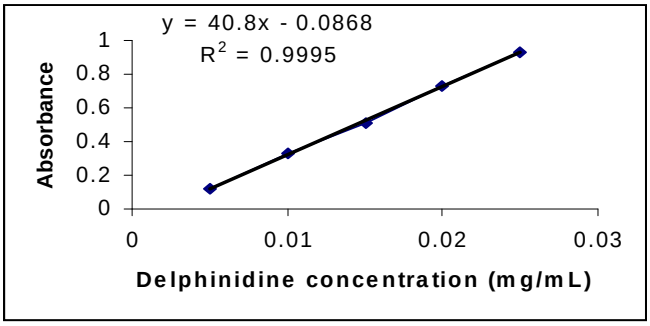

Figure 3. Calibration curve and its parameters.

The total anthocyan content in blubbery fruits was determined based on the extracts absorbance and calibration curve (Table 2).

Table 2.

Experimental data used for determination of total anthocyan content.

Extract	Absorbance	Concentration (mg/ml)	Total anthocyan content (mg/g)
A	0.350	0.0107	21.4
B	0.740	0.0202	40.4
C	0.684	0.0189	37.8
D	0.687	0.019	38.0
E	0.643	0.0179	35.8

From quantitative data presented in tables 1 and 2 we can say that the content of anthocyan and delphinidine is correlated with the extraction techniques used. The biocompound extracted quantities using reflux, sonication and microwave as extraction techniques are comparable. The prolonged heating and thermolability of the anthocyan can explain the lower extraction efficiency of the Soxhlet techniques. Centrifugation at room temperature provides the better extraction efficiency than other tested techniques.

IV. CONCLUSIONS

From this experiment we can conclude that the extraction composition does not depend upon the extraction techniques used. The extracted biocompound quantities are depending upon the extraction techniques involved. The best extraction technique is centrifugation at room temperature.

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