SOLVATOCHROMIC PARAMETERS OF SOME ANTHOCYANIN DERIVATIVES CONCENTRATED FROM SELECTIVE NATURAL EXTRACTS

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Anthocyanins are natural compounds with multiple functions and protective properties for both plants and other organisms. As natural pigments they exhibit excellent solvatochromic properties, in controlled purity conditions and under constant experimental and conservation conditions such as working temperature or pH value. This paper presents the experimental procedures related to the following objectives: obtaining the selective natural extracts of anthocyanins, glucoside derivatives of pelargonidin respectively, by solid-liquid and liquid-liquid extraction; purifying the resulted extract using adequate chromatographic methods; characterization of the quality of the concentrates obtained by spectral and HPLC methods. Based on the spectral data, correlations were established between the solvatochromic properties and their polarity parameters.

Keywords: selective anthocyanins extracts, pelargonidin derivatives, polarity parameters, chemical shifts, positive sovatochromism

1. Introduction

Anthocyanins are natural compounds with multiple protective functions and properties for both plants and other organisms, such as UV protection properties for plants, the role of antioxidant agents and free antiradicals, as well as prevention of diabetes and tumours. Pelargonidin is one of the most representative compounds of this class and it is present in natural compounds in the form of pelargonidin 3-O-β-glucopyranoside (Fig.1) or pelargonidin 3,5-O-β-diglucopyranoside (Fig.2). These glycosides are found in: cherries, strawberries, geranium, blueberries, tomatoes, red beans, eggplant peel, etc.

The extraction procedure was performed in compliance with the literature data [1-4], through specific methods of solid-liquid and liquid-liquid extraction. The extraction and purification of the glycoside derivatives of pelargonidin were

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done in order to exhibit the solvatochromic effects in different solvents in relation to their polarity parameters.

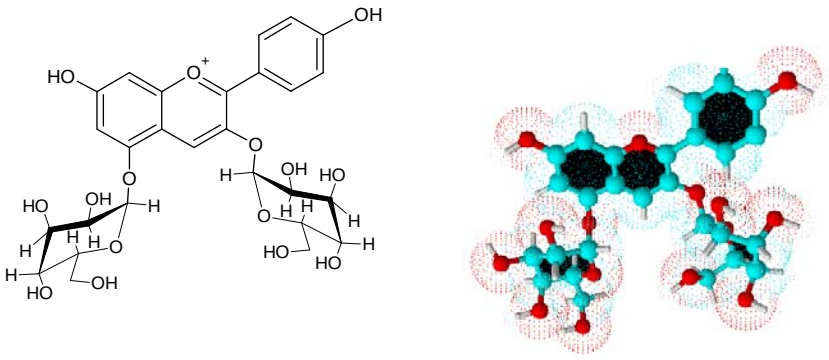

Fig. 1. Pelargonidin 3,5-O-diglucoside

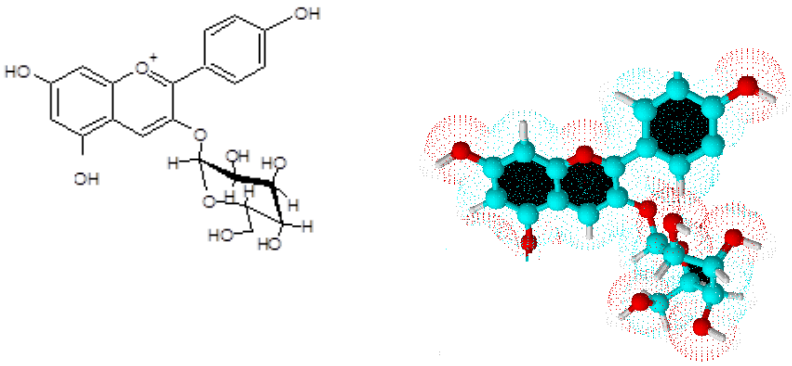

Fig. 2. Pelargonidin 3-O-glucoside

2. Experimental

Materials and method

The vegetal materials used in this work were geranium flower petals (Pelargonium zonale). The solvents were of chromatographic purity: methanol, ethanol, 1-propanol, 1-butanol, cyclohexanol. During all the experimental determinations, the pH was kept at the value of pH=2, with the addition of a HCL 1M solution, in order to preserve the structure of flavylium ion. The solid extract was redissolved in ethanol of chromatographic purity in order to determine the composition by HPLC.

The equipment used for the HPLC analysis was the Thermo Finnigan Surveyor Thermo Fisher Scientific Inc. (Whatman, USA) chromatographic system and the separation was made by reversed phase Aquasil C18 column (5μm, 250 x 4,6 mm) with elution gradient: water/formic acid/acetonitrile. The procedure of chromatographic analysis was carried out under the conditions described in literature [5, 11]. The mobile phase was represented by two eluents: solvent A: water/formic acid/acetonitrile in volume percent 87:10:3 and solvent B: water/formic acid/acetonitrile in volume percent 40:10:50, with eluent B gradient increasing from 6% to 60%. The detection was spectrophotometric at a wavelength of 518 nm. The injection volume of the extract in ethanol was 25 μL, the mobile phase flow rate was 0.8 mL/min.,the column temperature was 40°C, the work duration: 55 min.

The absorption spectra of pelargonidin, with the same concentration c = 0.2 mg/mL, were recorded in the 450-800 nm spectral domain, in 1 cm cuvettes, using the Specord S 600 spectrophotometer.

Solvents with different polarities are represented by the following ternary systems (ST):

ST1. Anthocyanin – water – methanol
ST2. Anthocyanin – water – ethanol
ST3. Anthocyanin – water – 1-propanol
ST4. Anthocyanin – water – 1-butanol
ST5. Anthocyanin – water – cyclohexanol.

3. Results and discussion

3.1. HPLC analysis

The chromatogram of pelargonidin concentrate extract is illustrated in Fig. 3. The following components can be identified from the chromatogram:

- the main component is represented by pelargonidin chloride (at a retention time of 31,813 min.);
- malvidin-3-acetylglucoside (34,517 min.) with a weight of aprox. 50% in the concentration of the main component;
- delphinidin-3-glucoside (15,175 min.) with a weight of aprox. 18% in the concentration of the main component;
- cyanidin-3-glucoside (17,318 min.) with a weight of aprox. 17% in the concentration of the main component;
- malvidin-3-cumarylglucoside (39,910 min.) with a weight of aprox. 9% in the concentration of the main component.

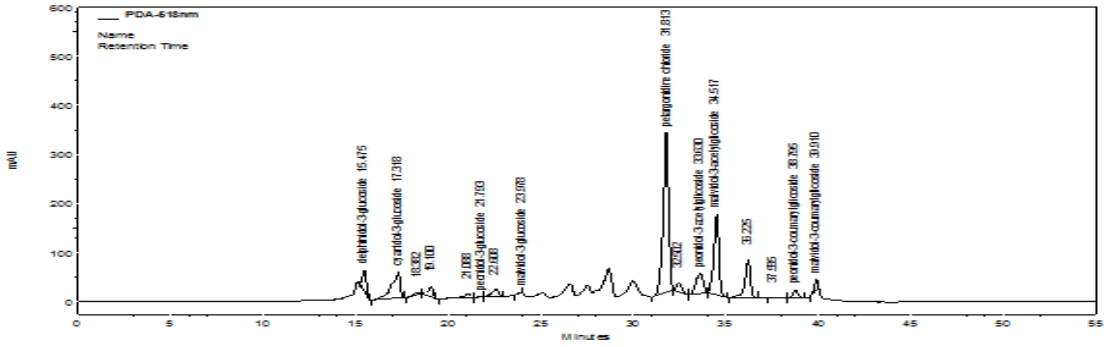

Fig. 3. HPLC chromatogram of the concentrate extract of pelargonidin

1.1.Solvatochromic effect

A series of pelargonidin concentrates were initially prepared by dissolving the concentrate in the same amount of solvent binary mixture consisting of alcohol - water at different proportions represented in Table 1.The molecular absorption spectra recorded in the visible domain are presented in fig.4.

Table 1

Composition of the analysed ternary systems: ST1-ST7

Concentration	Solution 1 water	Solution 2 ST1	Solution 3 ST2	Solution 4 ST3	Solution 5 ST4	Solution 6 ST5
Water (%)	100	25	25	25	25	25
Methanol (%)	0	75				
Ethanol (%)	0		75			
1-propanol (%)	0			75		
1-butanol (%)	0				75	
Cyclohexanol	0					75
Anthocyanin (mg mL^{-1})	0.2	0.2	0.2	0.2	0.2	0.2

The solvent effect produced by these ternary systems is determined by the polarity parameters of the contained alcohols, presented in Table 2 [10].

Solvatochromism is, in a narrow sense, the ability of a chemical substance to present different colours due to the change of the solvent in the solution. In a wider sense, solvatochromism can represent the spectral modifications of the absorption bands produced by the same cause. The hypsochrome chemical shifts recorded for the chromophore substances dissolved in solvents with an increasing

polarity correspond to a negative solvatochromism, while the bathochromic chemical shifts recorded for the dissolution in solvents with decreasing polarity correspond to a positive solvatochromism.

Table 2

The polarity parameters of the alcohols used as solvents

Nr.	Alcohol	Z (kcal/mol)	E_T (30) (kcal / mol)	E_T^N
1	Methanol	83.6	55.4	0.762
2	Ethanol	79.6	51.9	0.608
3	1-Propanol	78.3	50.7	0.617
4	1-Butanol	77.7	50.2	0.602
5	Cyclohexanol	-	46.9	0.500

The main goals of the spectral study were to identify the chemical shifts produced by the differentiated polar nature of a specially prepared series of solvents in which the solid extracts have been redissolved.

The quantitative modelling of the structure-activity relationship (QSAR) for anthocyanins can provide very useful information regarding their antioxidant action, in the form of structural and local parameters. Thus, based on the statistic equations and the prediction capacity of the modelling methods, there have been reported [5] values of the antiradical activity: $R_2 = 0.927$, $Q_2 = 0.871$ and of the antioxidant activity: $R_2 = 0.901$, $Q_2 = 0.841$.

The effect of the solvent was quantified by the evaluation of empirical polarity parameters with the following significance:

Z = The empirical parameter of the solvent polarity, determined on CT absorption of pyridinium iodide. Reichard defines [6] this parameter as transition molar energy, ET, expressed in kcal/mol for the load transfer band of 1-ethyl-4-methoxycarbonylpiridinium iodide in different solvents:

$Z = ET\ (kcal/mol) = h \bullet c \bullet \square\nu \bullet NA = 2{,}859 \bullet 10\text{-}3 \bullet \square\nu\ cm^{-1}$

$Z = ET\ (kj/mol) = h \bullet c \bullet \square\nu \bullet NA = 1{,}196 \bullet 10\text{-}2 \bullet \square\nu\ cm^{-1}$

$\square\nu$= wavenumber,

h= Planck constant,

c= speed of light,

NA= Avogadro's number

Dimroth and Reichardt [7] proposed another empirical parameter for solvent polarity: ET(30), which is based on the transition energy for the furthest solvatochromic wavelength of the absorption band for the betaine colorants, based on piridium-N-fenoxid. The size of this energy is expressed in kcal/mol. Therefore a new form of energy is used, called normalized energy, ETN, defined by the following expression:

$ETN = (ET_{(solvent)} - ET_{(TMS)}) / (ET_{(water)} - ET_{(TMS)}) = (ET_{(solvent)} - 30,7) / 32,4$

where: $ET_{(solvent)}$ = transition energy for the investigated solvent, $ET_{(TMS)}$ = transition energy for the standard: tetramethylsilane TMS, $ET_{(water)}$ = transition energy for water.

The absorption spectra of the pelargonidin extract exhibit chemical shifts of the maximum absorption bands differentiated for the 7 ternary systems, Figs. 4 and 5, as shown by the data in Table 3.

Table 3

Correlation of the spectral data of the pelargodinin extract with the polarity parameters of the alcohols from the solvent composition

Solution	Z (kcal/mol)	$E_T(30)$ (kcal / mol)	E_T^N (kcal / mol)	A	λ (nm)
1 (Water)	94,6	63,1	1,000	0,254	513,6
2 (ST1)	83,6	55,4	0,762	0.475	514,2
3 (ST2)	79,6	51,9	0,608	0,430	518,2
5 (ST3)	78,3	50,7	0,617	0,506	519,7
6 (ST4)	77,7	50,2	0,602	0,558	520,4
7 (ST5)	-	46,9	0,500	0,432	523,4

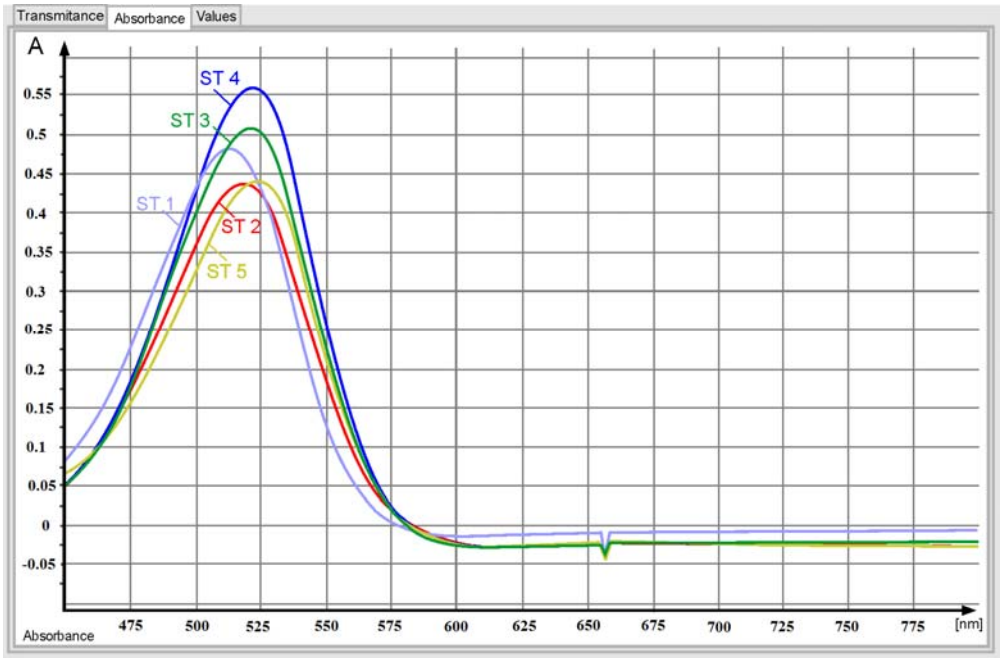

Fig. 4. Chemical shifts for ternary systems with different polarities: ST1: anthocyanin – water-methanol; ST2: anthocyanin – water – ethanol; ST3: Anthocyanin – water – 1-propanol; ST4: Anthocyanin – water – 1-butanol; ST5: cyclohexanol

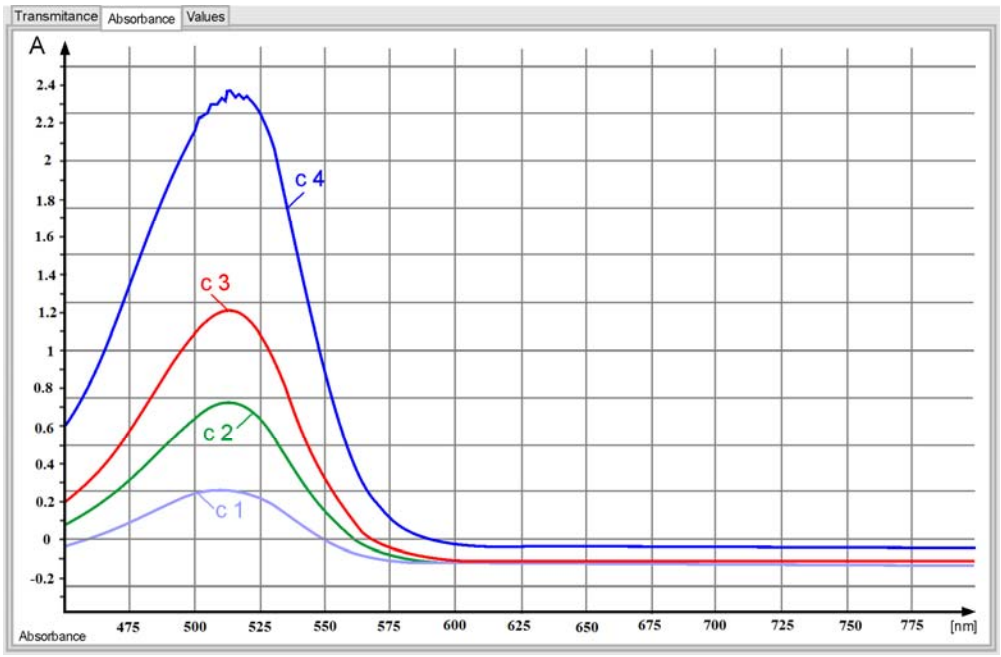

Fig. 5. Spectra of the pelargonidin extract redissolved in water, at different concentrations: c_1= 0.2mg/mL; c_2= 0.4mg/mL; c_3= 0.6mg/mL; c_4= 1.2mg/mL

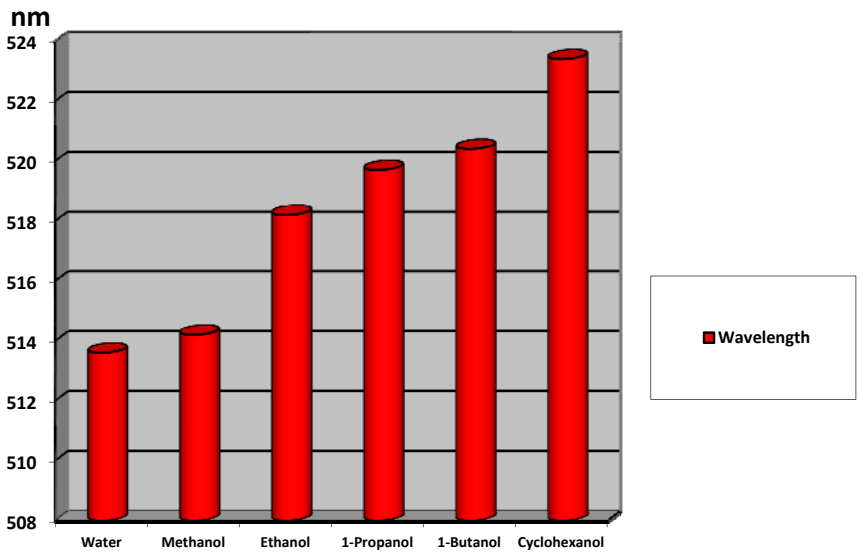

Fig. 6. Variation of the wavelength of the maximum absorption bands, for the pelargonidin extract, in solvents with different polarity (bathochromic effect recorded when reducing polarity)

The correlation of the spectral data of the pelargodinin extract, dissolved in the six solvents, with the alcohols polarity parameters shows a relative linearity. The correlation coefficients for the dependence of the wavelength of maximum absorption and the three parameters of solvent polarity: Z, ETN , ET (30) are exhibited in Table 4.

Table 4

Correlation of the spectral data of the pelargodinin extract with the polarity parameters

Nr	Dependence	r
1	$\lambda = f_{(Z)}$	0,85408
2	$\lambda = f_{(ET\ (30))}$	0,91404
3	$\lambda = f_{(ETN)}$	0,89830

If the water solvent (the most polar) and the cyclohexanol (the most non polar) are eliminated from the solvents a perfect correlation is obtained, r = 0,99942, of the spectral data and the empirical parameter of solvent polarity Z, (fig. 7.)

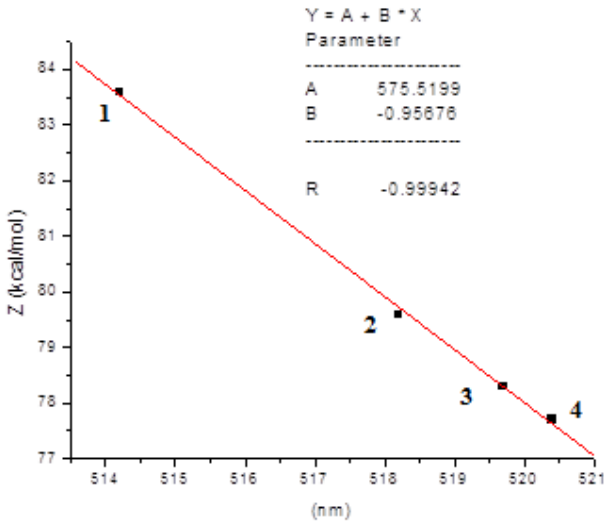

Fig. 7. Variation of the wavelength of the maximum absorption bands, for the pelargonidin extract, in methanol (1), ethanol (2), 1-propanol (3), and 1-buthanol (4)

4. Conclusions

The selective extracts obtained, concentrated and purified by chromatographic methods presented constant properties, specific to the

anthocyanin compounds which have been verified by spectrophotometric methods. In the case of spectra recorded for the flavylium ion, corresponding to pelargonidin, different chemical solvent effects were shown when dissolving in different solvents with differentiated polarity parameters, under the form of specific chemical shifts. When decreasing the polarity, the bathochromic effect recorded was inversely proportional with the value of the empirical parameter corresponding to the polarity of the solvent ET(30), or the value of the normalized energy ETN (negative solvatochromism). This effect corresponds to a positive solvatochromism – red shift.

The selective extracts of anthocyanin, advanced purified, prove to be useful in the study of the solvent effect or solvatochromism.

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