# GRAPE IVY (*PARTHENOCISSUS TRICUSPIDATA*) EXTRACTS WITH BIOACTIVE COMPOUNDS

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*The purpose of this paper is to present the results obtained regarding the extraction and characterization of the grape ivy (Parthenocissus tricuspidata) extract. Its fruits, grapes, are intensively colored and their skin was used to obtain a colored extract rich in anthocyanidins. HPLC, Liquid chromatography - mass spectrometry (LC-MS) and UV-VIS spectrophotometry were employed to characterize and identify the main extract compounds. The total polyphenol content was determined using the Folin Ciocalteu method while the total flavonoid content was spectrofotometrically evaluated. Additionally, the solvatochromic effect was investigated using different solvents. To highlight the fluorescence of the extract qualitative investigations were performed.*

**Keywords**: *Parthenocissus tricuspidata*, LC-MS, total phenolic content, fluorescence

## 1. Introduction

Many plants and fruits possess polyphenols, flavonoids and antioxidant activities and many researches were performed in order to find applications and to highlight the richness of the nature [1 – 3].

*Parthenocissus tricuspidata* (grape ivy) is a wild climbing plant belonging to grapes family. It can climb with its adhesive discs on different substrates, such as stone mountains, stone banks at the roadside, the exterior walls of buildings, thus withstanding strong winds and storms without detachment [4].

Usually the polyphenols and other valuable compounds are concentrated in fruits and therefore an extraction procedure has to be employed. There are several methods/techniques used for the extraction of the interest compounds.

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The most convenient extraction method is solid phase extraction (SPE), because it has many advantages: it is simple, efficient and it does not involve any sophisticated equipment, being therefore a low-cost method [5]. The purification mechanism includes interactions between the nonpolar stationary phase and a mobile polar phase [6] and lead to an anthocyanidin-rich fraction.

He and Giusti, in 2011, developed a mixed mode between cation exchange and reverse phase interactions in order to isolate anthocyanins from edible sources. According to their method the anthocyanins purification takes place using two different cartridges Amberlite XAD-7 and Sephadex LH-20, the last one being used in order to separate the anthocyanins from the proanthocyanidins [5]. However one of the cheapest extraction methods is maceration as it does not involve high quantities of solvents or complicated equipments.

The purpose of this study was to investigate the total content of polyphenols and flavonoids and the antioxidant capacity of the *Parthenocissus tricuspidata* fruit skin extracts as well as the spectral properties.

## 2. Materials and methods

### 2.1. Raw materials

The plant raw material used in this study is represented by the skins of the fruit of Parthenoccius tricuspidata (grape ivy). These fruits were collected in September 2019, from the Campulung Muscel area, Romania. The plant was grown in natural conditions without any changes.

For extraction and different measurement performed for the extract characterisation the following reagents were used: methanol (Merck), absolute ethanol (Merck), isopropanol (Sigma Aldrich), HCl 37% (Merck), FolinCiocalteu reagent (Sigma Aldrich), gallic acid (Sigma Aldrich), sodium nitrite (Merck), aluminium chloride (Merck), sodium hydroxide (Merck), quercetin (Sigma Aldrich), trolox (Sigma Aldrich), DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma Aldrich) and formic acid (Merck).

### 2.2. Preparation of the hydroalcoholic extract

The extraction procedure used was maceration. The fruit tails were removed and the skins were separated from the pulp to remove the carbohydrates. They were washed very well with cold water (1°C) to preserve the anthocyanidin content. The skins were dried at 25 °C for 36 hours in the dark.

On 30 grams of dry and crushed skins were added 250 mL of absolute ethyl alcohol. The mixture was left to soak in the dark for 7 days, at a temperature of 4-8°C, performing the homogenization operation daily. For a better extraction of anthocyanins, few drops of HCl 37% were added in order to have an acidic medium. After 7 days, the crude extract was filtered and thus a hydro-alcoholic extract rich in anthocyanins **without** vegetable residues was obtained.

### 2.3. Quantitative UV-Vis spectrophotometric measurements

**Total polyphenol content:** The method described by Ainsworh E. et al., 2007 was used to determine the total polyphenol content [7]. To determine the total polyphenol content, the ethanolic extract of *Parthenoccius tricuspidata* was mixed with 100 µL Folin-Ciocalteu reagent and left to stand for 5 minutes at 25 °C before adding the rest of the reagents. The samples were kept in the dark for 120 minutes before measuring the absorbance with the SPECORD 250 PLUS spectrophotometer at 765 nm. Gallic acid was used as a standard in a linearity range of 0-1250 mg $L^{-1}$ and the results were expressed as mg gallic acid equivalents / 100 g of fresh *Parthenocissus tricuspidata* skins.

**Total flavonoid content:** The total flavonoid concentration was calculated based on the reaction between 5% sodium nitrite, 10% aluminium chloride and 1 M sodium hydroxide using the method proposed by Singleton [8] with slight modifications. The absorbance was measured with the SPECORD 250 PLUS spectrophotometer at 430 nm. Quercetin was used as a standard in a linearity range of 0-125 mg $L^{-1}$ and the results were expressed as mg quecertin equivalents / 100 g of fresh *Parthenocissus tricuspidata* skins.

**Antioxidant capacity - DPPH Method:** DPPH free radical scavenging assay was used for the determination of antioxidant capacity (AC) of the extract. Absorbance measurements are transformed to antioxidant capacity using trolox as reference. In this assay, 6 mL of 0.09 mg/mL DPPH (2,2-diphenyl-1-picrylhydrazyl) methanolic solution was mixed with 0.5mL aliquots of appropriately diluted extract in distilled water and the absorbance was measured at 517nm, against methanol as blank, after 20 min at room temperature. Calibration was performed using trolox as standard, in the concentration range of 50-1000µmoli/L, and the antioxidant capacity was expressed as mmol/L Trolox equivalents / 100 g of fresh *Parthenocissus tricuspidata* skins.

### 2.4. Qualitative HPLC-PDA analysis of anthocyanins

For qualitative anthocyanins identification the MA-E-AS315-11-ANCYAN method proposed by the International Organisation of Vine and Wine (OIV) described in Compendium of International Methods of Analysis [9] was used. HPLC measurements were performed using a Thermo Finnigan Surveyor Plus system with a diode array detector. The chromatographic separation was performed using an Aquasil C18 (250 x 4.6 mm) column, operated at 40 °C and a solvent gradient as presented in reference [10]. UV spectra were recorded from 220 nm to 800 nm. The anthocyanins separated on the C18 column were identified based on elution order presented in OIV method.

### 2.4. UHPLC-MS/MS analysis

LC-MS measurements were performed using the Q-Exactive Focus High Resolution Mass Spectrometer (Thermo Fisher Scientific) equipped with heated electrospray ionisation (HESI) probe (Thermo Fisher Scientific). Separations were performed on Kinetex (100 mm × 2.1 mm, 1.7 μm) column using a binary solvent system: solvent A (water with 0.1% formic acid) and solvent B (methanol with 0.1% formic acid). The UHPLC gradient for mass screening was: 0 min, 2% B, 0–2 min, 2% B; 2–5 min, 2-50% B; 5–17 min, 50-98% B; 17-18 min, 98-2% B; 18–20 min, 2% B for final washing and equilibration of the column for the next run. The flow rate was 0.3 mL/min between 5-17 minutes and 0.4 mL/min otherwise; and the injection volume was 10 μL. The mass spectrometer was operated in negative mode following the ionization parameters optimized previously [11].

The identification and quantification of phenolic acids and flavonoids in the extract was performed according to their spectral characteristics: mass spectra, accurate mass, mass fragments and characteristic retention time, against external standard solutions analysed under the same conditions. For confirmation, fragmentation studies were performed by a data‑dependent scan with collision‑induced dissociation (CID) with normalised collision energy of the CID between 30 and 80 eV, depending of each phenolic compound. Data acquisition and data analysis were performed using Xcalibur software (Version 4.1) and ChemSpider (www.chemspider.com) was used as a reference library to identify compounds of interest.

**Fluorescence measurements**

The qualitative fluorescence measurements were performed firstly using a laser excitation at two wavelengths: $\lambda 1 = 550$ nm and $\lambda 2 = 480$ nm . More detailed fluorescence spectra were obtained using the equipment Duetta Fluorescence and Absorbance Spectrometer, producer by Horriba.

## 3. Results and discussions

### 3.1. Determination of the total content of polyphenols, total flavonoids and antioxidant capacity of fresh Parthenocissus tricuspidata skins

In table 1 are presented the results obtained for the extract of *Parthenoccius tricuspidata* in comparison with literature data related to different grapes genotypes. The total polyphenols content of *Parthenocissus tricuspidata* skins (2.228 mg/g) was lower compared with total polyphenols content of Vitis vinifera varieties at phenolic maturity, such as Cabernet Sauvignon (20 mg/g), Feteasca Neagra (12.5 mg/g), Mamaia (6.7 mg/g), Merlot (9.7 mg/g) and Pinot Noir (20.2 mg/g) [12] while the total content of flavonoids obtained for

*Parthenocissus tricuspidata* skins is of the same order of magnitude with that found in different varieties of red grapes [13].

A different behavior was observed related to the antioxidant capacity which was found ten times higher for *Parthenocissus tricuspidata* skins (20.3 mmol trolox equivalents /100 g) with respect to the antioxidant capacity of skin extracts of red grape (Vitis vinifera) varieties (table 1) [14]. All these results show that the fruits of ivy grape could represent a potential source of polyphenols and antioxidants.

*Table 1*

**The total content of polyphenols, total flavonoids and antioxidant capacity of the studied extract compared to skin extracts of different grapes genotypes**

| Polyphenols Total Content (mg galic acid equiv./100g) | | Flavonoids Total Content (mg quercetin equiv./100g) | | Antioxidant capacity (mmoli trolox equiv./100 g) | |
|---|---|---|---|---|---|
| Genotype | Value | Genotype | Value | Genotype | Value |
| *Parthenocissus tricuspidata* | 222.8 | *Parthenocissus tricuspidata* | 33.0 | *Parthenocissus tricuspidata* | 20.3 |
| Cabernet Sauvignon | 2000 | Banqui Abyad | 41.47 | Cabernet Sauvignon | 2.03 |
| Feteasca Neagra | 1250 | Black Muscat | 44.49 | Isabel variety | 3.64 |
| Mamaia | 670 | Pearl of Csaba | 47.05 | Sanglovese | 1.47 |
| Merlot | 970 | Cardinal | 66.50 | Negro Amaro | 1.30 |
| Pinot Noir | 2020 | Ruby Red | 68.66 | Pinot Noir | 1.11 |

### 3.2. HPLC-PDA qualitative identification of anthocyanins

Fig. 1 presents the HPLC chromatogram of the obtained extract. As it can be observed, the main anthocyanins identified in the extract were delphinidol 3-glucoside and malvidol 3-glucoside, followed by cyanidol 3-glucoside and petunidol 3-glucoside. Also, very small amount of peonidol 3-glucoside was observed.

Acylated and coumarylated glucosides of peonidin and malvidin were not identified, but at retention time of 11.173 was observed a peak with an intensity similar with that of delphinidol-3-glucoside, which could be malvidin-3,5-diglucoside, an anthocyanin characteristic to hybrid grape varieties and which is not present in Vitis vinifera species.

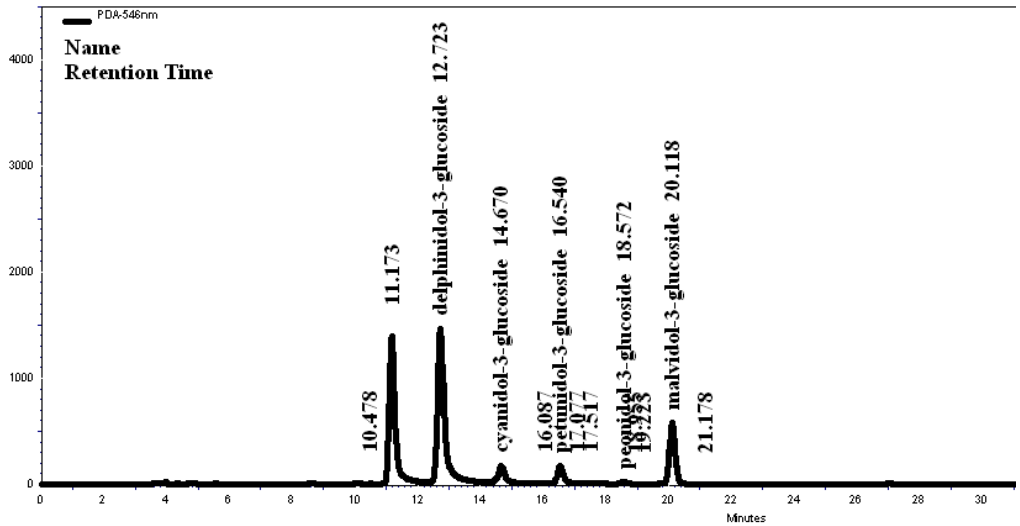


Fig.1. HPLC chromatogram of the extract obtained from the skins of *Parthenocissus tricuspidata* in ethanol

Future studies will be performed in order to identify this compound with accuracy (comparison with an analytical standard or LC-MS analysis).

### 3.3. LC-MS quantitative analysis of some phenolic acids and flavonoids

Table 2 presents the main phenolic acids and flavonoids quantified in the Parthenoccius tricuspidata ethanolic extract and skins by UHPLC-MS/MS analysis.

*Table 2*

**UHPL-MS/MS analysis of ethanolic extract Parthenoccius tricuspidata**

| Compound name | Chemical Formula | Exact mass[a] [M - H]− | Accurate mass [M - H]− | Concentration (µg/L extract) | Concentration (µg/100 g of fresh Parthenocissus tricuspidata skins) |
|---|---|---|---|---|---|
| Caffeic acid | $C_9H_8O_4$ | 179.0349 | 179.0342 | 160.7 | 133.92 |
| Epicatechin | $C_{15}H_{14}O_6$ | 289.0717 | 289.0719 | 15.6 | 13.00 |
| Chlorogenic acid | $C_{16}H_{18}O_9$ | 353.0877 | 353.0873 | 1.3 | 1.08 |
| Syringic acid | $C_9H_{10}O_5$ | 197.0455 | 197.0450 | 3847.3 | 3206.08 |
| Gallic acid | $C_7H_6O_5$ | 169.0142 | 169.0133 | 279.3 | 232.75 |
| Quercetin | $C_{15}H_{10}O_7$ | 301.0353 | 301.0356 | 864.7 | 720.58 |
| Kaempferol | $C_{15}H_{10}O_6$ | 285.0404 | 285.0406 | 25.8 | 21.50 |

[a] Calculated mass of the parent ion using free chemical database, ChemSpider

As it can be observed, the extract contains important amounts of syringic acid (3847.3 mg $L^{-1}$ extract) and quercetin (864.7 mg $L^{-1}$ extract) and also considerable amounts of gallic acid (279.3 mg $L^{-1}$) and caffeic acid (160.7 mg $L^{-1}$ extract). Comparing with grapes skins of Vitis vinifera species (eg. Cabernet Sauvignon, Feteasca Neagra, Mamaia, Merlot, Pinot Noir), the *Parthenoccius tricuspidate* skins contains about ten times more syringic acid and epicatechin [12].

### 3.4. Determination of fluorescent properties

In order to study the solvent influence on the *Parthenoccius tricuspidata* extract **t**he qualitative fluorescent properties of the extracts were checked firstly by laser excitation at two wavelengths 550 nm and 480 nm and the visual observations reveal a visibil fluorescence.

The Horiba Duetta Spectrofluorimeter is a 2-in-1 equipment (fluorescence and absorbance spectrometer) thus it can simultaneously measure the fluorescence and the excitation spectra. The recorded spectra for the extracts obtained in ethanol and isopropanol are presented in Fig. 4.

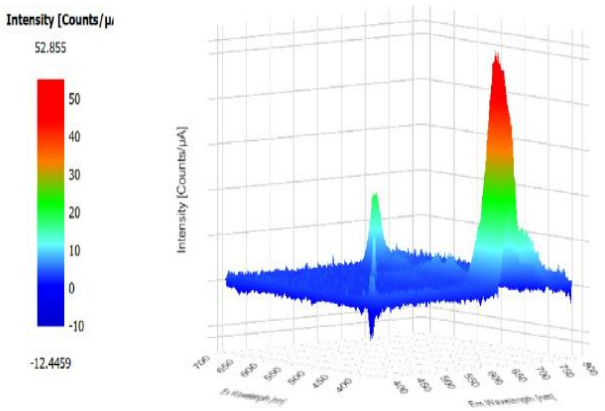


Fig. 4.a. The emission (300-800 nm) and excitation (250-700nm) spectrum of Parthenoccius tricuspidata extract in ethanol

It can be seen the important fluorescence obtained for the extract in isopropanol. The wavelength at which the maximum absorption was recorded is 540 nm in the case of ethanol extract and 546 nm in the case of isopropanol extract, while the maximum emission was observed at 587 nm for the ethanolic extract and 593 nm for the extract obtained in isopropanol.

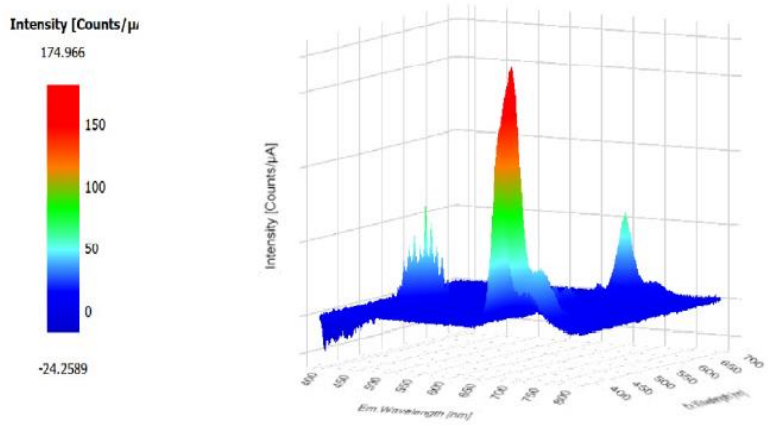


Fig. 4.b. The emission (350-800 nm) and excitation (300-700nm) spectrum of the Parthenoccius tricuspidata extract in isopropanol

It can be concluded that the *Parthenoccius tricuspidata* extract presents fluorescence and the polarity solvent has a solvatochromic effect (Bathocromic effect on polarity decreasing). This conclusion was confirmed also by the results regarding the water extract. From Fig. 5 it can be seen that increasing the solvent polarity the maximum wavelength is shifted towards higher energies (lower wavelength). The results are comparable with those obtained by S.D. Iosub et al. for the pelargonidin extract [15].

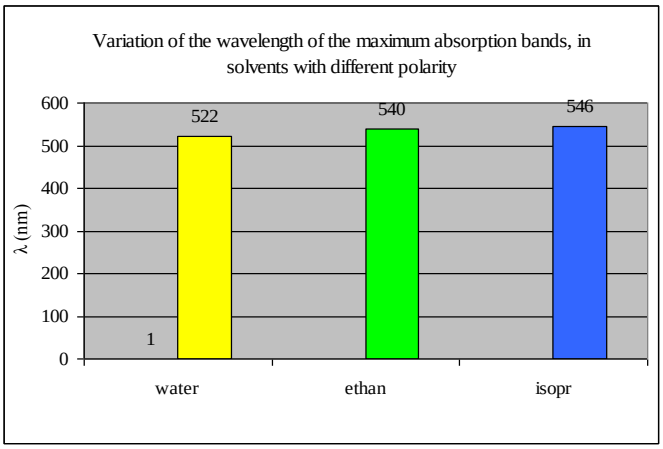


Fig. 5. Variation of the wavelength of the maximum absorption bands, for P. Tricuspidata extract, in solvents with different polarity (bathochromatic effect on polarity decreasing)

## 6. Conclusions

In sumary, using the maceration technique fluorescent alcoholic *Parthenoccius tricuspidata* extract rich in antocyanins and flavonoids compounds can be obtained. The fingerprints resulting from the LC-MS analysis allowed the identification of phenolic acids (caffeic acid, chlorogenic acid, syringic acid), flavonoid aglycones (kaempferol, quercetin), while HPLC analysis put in evidence the main anthocyanin compounds: delphinidol 3-glucoside, malvidol 3-glucoside, petunidol 3-glucoside, peonidol-3-glucoside. The extracts present also fluorescence properties and the solvent used for extraction has spectral influence. It can be concluded that from *Parthenoccius tricuspidata* fruit skin can be obtained extracts with good antioxidant properties as well as optical properties, extracts which can find applications in different fields.

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## R E F E R E N C E S

[1]. *A, Bramorski, A. d. R. Cherem, C. P. Marmentini, J. Torresani, T. Mezadri, A. de.A. S. Costa*, Total polyphenol content and antioxidant activity of commercial Noni (Morinda citrifolia L.) juice and its components, Brazilian Journal of Pharmaceutical Sciences, **Vol. 46**, no. 4, Oct./Dec., 2010.

[2]. *N. Radu; M. Voicescu, E. Radu, C. Tanasescu*, Biomaterial with antioxidant and antifungal activities, obtained from romanian indigenous plants, Molecular crystas and liquid crystals, **Vol. 655**, Issue: 1, 2017, 243-249.

[3]. *L. Suwen, L. Xiaoying, G. Zhaoyuan, Z. Xuan, C. Xuedong*, Polyphenol Content, Physicochemical Properties, Enzymatic Activity, Anthocyanin Profiles, and Antioxidant Capacity of Cerasus humilis (Bge.) Sok. Genotypes, Journal of Food Quality, **Vol. 2018**, Article ID 5479565, 2018, 13 pages.

[4]. *H. Tianxian, L. Zhang and W. Deng* , Biological adhesion of Parthenocissus tricuspidata, College of Materials, Science and Engineering, South China University of Technology, Guangzhou 510640, PR China, Archives of Biological Sciences **Vol. 63,** Issue 2, 2011, 393-398.

[5]. *J. He and M.M. Giusti*, High-purity isolation of anthocyanin mixtures from fruits and vegetables – a novel solid-phase extraction method using mixed mode cation-exchange chromatography, Journal of Chromatography, 2011, 7914-7922.

[6]. *L.E. Rodriguez-Saona and R.E. Wrolsta*, Extraction, isolation, and purification of anthocyanins, Current Protocols in Food Analytical Chemistry, Hoboken, NJ, John Wiley & Sons, 2001, Inc.: F1.1.1-F1.1.11.

[7]. *E. Ainsworth, K. Gillespie*, Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat Protoc 2, 2007, 875–877.

[8]. *V. L. Singleton, J.A.Jr. Rossi*, Colorimetric of total phenolics with phosphomolybdic-phosphotungstic acid reagents, American Journal of Enology and Viticulture, 1965; **Vol. 16:** 144-158.

[9]. *OIV*, HPLC - determination of nine major Anthocyanins in red and rosé wines. In Compendium of international methods of analysis of wine and musts, International Organisation of Vine and Wine. **Volume 2**, 2008, 48–60.

[10]. *E. I. Geana, A. M. Iordache, R. E. Ionete*, Assessing the wine anthocyanin profile for red grape varieties identification, Progress of Cryogenics and Isotopes Separation, **Vol. 14**, issue 1/2011, 127-133.

[11]. *C.T Ciucure, I. E. Geană*, Phenolic compounds profile and biochemical properties of honeys in relationship to the honey floral sources. Phytochem. Anal. 30, 2019, 481–492. doi:10.1002/pca.2831.

[12]. *I. E. Geana, R.E. Ionete, V. Niculescu, V. Artem, A. Ranca*, Changes in polyphenolic content of berry skins from different red grapes cultivars during ripening, Pgress of Cryogenics and Isotopes Separation, **Volume 19**, issue 2/2016, 95-104.

[13]. *T. Sahoo, M.K. Verma, Kaluram*, Analysis of total flavonoids content in grape genotypes, international journal of chemical studies **Volume 7**, iss. 1, 2019, 1181-1184**.**

[14]. *I. I. Rockenbacha, L. Valdemiro, V. Gonzaga, M. R. Any, E. d. S. Schmidt, M. Gonçalvesb, I. Genoveseb, R. Fetta*, Phenolic compounds and antioxidant activity of seed and skin extracts of red grape (Vitis vinifera and Vitis labrusca) pomace from Brazilian winemaking, Food Research International, **Volume 44,** Issue 4, May 2011, 897-901.

[15]. *S. D. Iosub, A. Meghea, I. Geana*, Solvathochromic parameters of some anthocyanin derivatives concentrated from selective natural anthocyanin derivatives concentrated from selective natural extracts, U.P.B. Sci. Bull., Series B, **Vol. 76**, Iss.1, 2014.