Annals of the University of Oradea, Fascicle: Ecotoxicology, Animal Husbandry and Food Science and Technology, Vol. XVII/B 2018

Analele Universitatii din Oradea, Fascicula: Ecotoxicologie, Zootehnie si Tehnologii de Industrie Alimentara, Vol.XVII/B 2018

EVALUATION OF TOTAL ANTHOCYANINS EXTRACTED FROM *HIBISCUS* USING DIFFERENT WATER-BASED SYSTEMS

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Abstract

Hibiscus is a medicinal plant which contain a large amount of phytochemical compounds. The petals of Hibiscus flower are rich in anthocyanins, bioactive molecules with strong antioxidant properties. The purpose of the study was to comparatively evaluate the yield of extraction of anthocyanins from Hibiscus sabdariffa L. petals, using an eco-friendly technology based on mineral acidified and non-acidified water as extraction solvent compared to ethanol. Extraction was performed in waters with different mineral content and pH values ranging from 5 to 8, at 40°C. Quantitative analysis of anthocyanins was done using the pH differential spectrophotometric methods. The highest content of total anthocyanins was found in extracts with Borsec plain mineral water (597.960 mg/100g DM). Acidification with 1%, 2% and 3% acetic acid of the water- and ethanol-based solvents improved the extraction of anthocyanins. A strong negative correlation was found between pH values of water-and ethanol-based extraction systems, and total anthocyanins content. No statistically significant correlation was found between dissolved minerals content in bottled water and total anthocyanins.The obtained extracts can be used as food ingredients or as ingredients intended for different applications such as pharmaceutical, dietary supplements or cosmetics.

Key words: *Hibiscus sabdariffa* L., water, ethanol, anthocyanins

INTRODUCTION

Hibiscus is a species of plant which attracts attention due to its composition rich in phytochemical compounds (Obouayeba A.P. et al. 2014) which are beneficial for the treatment or prevention of different diseases (Abba P. et al., 2014). The major compounds found in this plant are polyphenolic acids, flavonoids and anthocyanins (Carvajal Z.O. et al., 2012), such as delphinidin-3-O-glucoside, cyanidin-3-O-sambubioside and delphinidin-3-O-sambubioside (Formagio A. et al., 2015). The extracts of petals and sepals have different pharmacological properties (Olvera G. et al., 2008; Lin et al., 2005) such as antioxidant (Da-Costa-Rocha I. et al., 2014; Lee et al., 2002) and hepatoprotective (Abba P. et al., 2014; Okonkwo T.J.N., 2010), being used in traditional medicine mainly as diuretic and laxative (Monroy O. and Castillo E., 2007).

Anthocyanins are natural pigments found in various plants, known for their applications in food industry (Bridle & Timberlake, 1997). They

are water-soluble compounds with low to medium stability mostly under increased temperature, light and pH conditions (Claudia G.I. et al., 2017). The extraction of anthocyanins from the plant matrix is usually performed in organic solvents (acetone, methanol, ethanol) in particular with addition of low amounts of acids (HCl) which stabilizes the red flavylium cation (Giusti M.M. et al., 2001).

The aim of the present study was to investigate the influence of pH, mineral content and acidification of various types of water (well drinking, spring, deuterium depleted, mineral/sparkling and distilled) and ethanol solutions on the recovery of anthocyanins from *Hibiscus sabdariffa* L. petals.

MATERIAL AND METHOD

Plant material and reagents

Dry *Hibiscus sabdariffa* L. flowers were purchased from local market of natural products (Dacia Plant, Romania). Samples were grounded and stored at 4^{o}C until analyses. The moisture content was determined at 105 ℃ using the moisture analyser (MAC 210 - RADWAG, Poland).

Chemical reagents of analytical purity were used.

Extraction of anthocyanins

Extraction was initially performed in water of different sources (well drinking water, spring water, deuterium depleted water, mineral/sparkling from three companies, distilled water) at 40^{o}C for 24 hours. For comparison, 70% aqueous ethanol was used as extraction solvent. Further, 1%, 2% and 3% acetic acid acidification of the water system proved to be efficient in the first set of experiments, were tested as acid environment was shown to favorize flavylium cation form of anthocyanins.

The pH of different water-based and ethanol-based solvents was measured using the SevenCompact pHmeter (Mettler Toledo). The pH values of the extraction solvents investigated in the present study are shown in Table 1.

Table 1

pH values of water-based and ethanol-based extraction solvents used in the present investigation

Sample	Extraction solvent/ source	pH
1.	Well water/ Barcani, Romania	7.009
2.	Sulfur spring water/ Barcani, Romania	8.014
3.	Deuterium depleted water/ ICSI V âlcea, Romania	7.688
4.	Plain mineral water/ Borsec, Romania	7.525
5.	Sparkling bottled water/ Borsec, Romania	6.063
6.	Plain mineral water/ Dorna, Romania	7.737

7.	Sparkling bottled water/ Dorna, Romania	5.823
8.	Plain mineral water/ Apa Craiului, Romania	7.483
9.	Sparkling bottled water/ Apa Craiului, Romania	5.327
10.	Purified water (distilled)	6.653
11.	70%Ethanol	7.037
12.	Acidified 1% acetic acid Borsec Plain mineral water	3.239
13.	Acidified 2% acetic acid Borsec Plain mineral water	3.001
14.	Acidified 3% acetic acid Borsec Plain mineral water	2.846
15.	Acetic acid 1% in ethanol	3.738
16.	Acetic acid 2% in ethanol	3.609
17.	Acetic acid 3% in ethanol	3.391

Assay of total anthocyanins

The quantitative analysis of total anthocyanins was done using the pH differential spectrophotometric method (Schwartz S.J., 2001). The Specord 200Plus UV–Vis spectrophotometer (Analytik Jena, Germany) was used. The results were expressed as mg Cyn–3–O–G equivalents $100g^{-1}$ DM.

Statistical analysis

All of the measurements were performed in duplicate. Results were calculated and expressed as mean values of duplicate determination. The Pearson correlation coefficients were calculated from the data.

RESULTS AND DISCUSSION

The absorption spectrum of anthocyanins extracted from *Hibiscus sabdariffa* L. showing the maximum absorption band at 519 nm is presented in Figure 1.

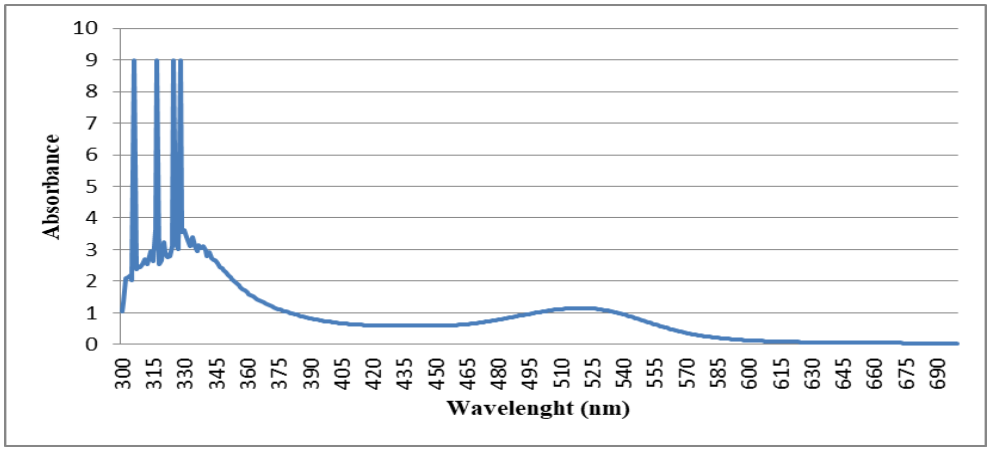

Fig.1. The absorption spectrum of *Hibiscus sabdariffa* L. water extract.

The chemical composition as declared by manufacturer of mineral/sparkling water used as solvent for eco-friendly extraction of anthocyanins from *Hibiscus sabdariffa* L. is presented in Table 2. As noticed, biominerals *e.g.* calcium, magnesium and sodium were present in highest amounts in all tested bottled waters.

Table 2

The chemical composition of mineral/sparkling water used for the extraction of *Hibiscus sabdariffa* L. anthocyanins

Type of water	**Chemical composition (mg/L)**							
	HCO_3^-	**Ca^{2+}**	**Mg^{2+}**	**Na^+**	**Cl^-**	**SO_4^{2-}**	**K^+**	**NO_3^-**
Plain mineral water (Borsec)	344.3	61.1	27.6	2.8	-	-	-	-
Sparkling bottled water (Borsec)	1874	382.4	101	90.2	-	-	-	-
Plain mineral water (Dorna)	201.3	65.19	1.48	0.72	1.06	6.17	0.46	-
Sparkling bottled water (Dorna)	195.2	76.95	1.81	0.86	1.1	5.9	0.38	-
Plain mineral water (Apa Craiului)	-	63.24	2.1	0.95	4.8	-	0.4	3.9
Sparkling bottled water (Apa craiului)	960	297	9.7	21.9	5.6	-	-	0.6

The results regarding the total anthocyanins of *Hibiscus sabdariffa* L. recovered from the different experimental runs are shown in Figure 2. Results were compared to 70% ethanol extracts.

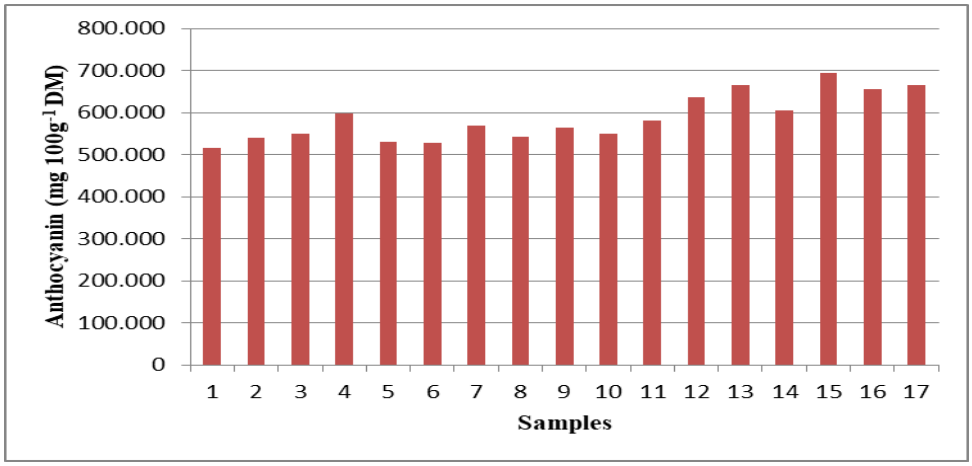

Fig. 2. Total anthocyanins content of *Hibiscus sabdariffa* L. extracted at 40°C in different water systems and acidied water systems.

The highest amounts of total anthocyanins were found in sample **4** (597.960 mg $100g^{-1}$ DM) extracted with Borsec plain mineral water. The content was higher than that of extract in 70% ethanol. The lowest content was registered in sample **1** (515.225 mg $100g^{-1}$ DM) extracted with well water/ Barcani, Romania.

No statistically significant correlation was found between water minerals and total anthocyanins in samples **4-9**. However, the most efficient extraction was obtained using plain bottled water from Borsec company, water which contains almost the smallest quantities of dissolved minerals compared to the other types of investigated water.

It was shown that acidified methanol and ethanol solutions with HCl enhanced the extraction of anthocyanins because of the presence of the red stable flavylium cation (Giusti M.M. et al., 2001). Considering these findings, acidification of Borsec plain mineral water and 70% ethanol with three different concentrations of acetic acid has been investigated. Acetic acid was selected as eco-friendly acid compared to mineral ones. The results (Figure 2) showed higher anthocyanins concentrations than those in non-acidified solvents in all samples (**12-17**). The highest content (693.440 mg/$100g^{-1}$ DM) was registered in sample extracted with 1% acetic acid in 70% ethanol, while the lowest one was found in acidified 3% acetic acid Borsec Plain mineral water (604.320 mg $100g^{-1}$ DM) . However, the results obtained in acidified waters were very close to those in ethanol, which means that water can be a good alternative of ethanol for *Hibiscus sabdariffa* L. anthocyanins extraction. Moreover, aqueous extracts enriched in minerals originating from selected waters may show increased health beneficial properties.

A strong negative correlation was found between pH values of water-and ethanol-based extraction solvents and total anthocyanins concentration (Pearson correlation coefficient R= −0.8204).

The obtained results showed eco-efficiency of water extraction of *Hibiscus* anthocyanins, values being comparable to those reported by other authors, Yin W. et al. (2012), who found a content of 155.28 mg $100g^{-1}$ DM in 99,7% ethanol and 205.76 mg $100g^{-1}$ DM in aqueous extracts.

CONCLUSIONS

Various well drinking, spring, deuterium depleted, mineral/sparkling and distilled waters investigated for the eco-friendly extraction of anthocyanins from *Hibiscus sabdariffa* L. showed efficiency when compared to ethanol. The highest content of total anthocyanins was found in extracts with Borsec plain mineral water (597.960 mg $100g^{-1}$ DM).

Acidification with 1%, 2% and 3% acetic acid of the water- and ethanol-based solvents improved the extraction in all experimental runs.

A strong negative correlation was found between pH values of water-and ethanol-based extraction solvents and total anthocyanins concentration. No statistically significant correlation was found between dissolved minerals content in bottled water and total anthocyanins.

The obtained results described the simple and efficient green extraction of *Hibiscus* anthocyanins in water of different mineral composition and pH, at 40^{o}C, leading to a safe extract which can be used in various applications.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian National Authority for Scientific Research –UEFISCDI, project number PN-III-P2-2.1-CI-2018-1401.

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