THE TOTAL ANTHOCYANINS CONTENT OF *HIBISCUS* SPECIES

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Abstract

Hibiscus is an evergreen herbaceous plant which belongs from Malveceae family and it contains several hundred species through the world. The aim of our study was to investigate the total anthocyanins content of three species of *Hibiscus* (*H. sabdariffa and Hibiscus syriacus*, blue and yellow*)* in order to use it in the future in the field of dermatology and pharmacology. Between these three species of *Hibiscus* considered by this study, the one with the highest anthocyanins content was *H. sabdariffa* (361.37±5.32 mg cyaniding-3-glucoside/100 g). Knowing the bioactive compounds from plant extracts, these can be used in the desired direction with maximum efficiency.

Key words: anthocyanins, *Hibiscus*, UV-Vis spectroscopy

INTRODUCTION

Hibiscus is an evergreen herbaceous plant which belongs from Malveceae family. It contains several hundred species through the world. A high number of research studies showed that flower of *Hibiscus* ssp. has various pharmacological properties. The bioactive compounds from *Hibiscus* flower belongs from flavonol, anthocyans, and phenolic acid classes (Kumar & Singh, 2012).

Hibiscus sabdariffa L. widely grown in Malaysia and other countries such as Indonesia, Africa and America and its calyxes were deeply red in color due to the presence of anthocyanins compound; delphinidin-3-sambubioside, cyanidin-3-sambubioside, cyanidin-3-glucoside and delphinidin-3-glucoside which are the non-methylated type (Aishah et al., 2013).

Hibiscus syriacus L. is widely distributed all around the world as ornamental and green plants and it is a medicinal plant used as antipyretic, antihelminthic, antifungal and antioxidant capacity due to the presence of the bioactive compounds from the petals. The main components of flower pigments are phenolic compounds, in particular, flavonoids and anthocyanin (Geng, et al 2012).

The aim of our study was to compare the content in total anthocyanins from three species of *Hibiscus* (*H. sabdariffa, H. syriacus,* yellow and blue*)* in order to use them in the future in the field of dermatology and pharmacology.

MATERIAL AND METHOD

Plant Material

Hibiscus syriacus (Gold Dust ,Yellow) codified HIB_yellow, *Hibiscus syriacus* (Oiseau Bleu or Blue Bird, Blue) codified HIB_blue and *Hibiscus sabdariffa* (Red) codified HIB_red, were used to analyses the content in total monomeric anthocyanins. In the Figure 1 are show the *Hibiscus* species used. The dried calyces of *Hibiscus sabdariffa* were purchased from a local market from Oradea, Romania. The petals of *Hibiscus syriacus,both* from blue colour were collected from the public gardens in the summer of the 2014 and the yellow colour from personal collection. All the the plants were identified at the Agriculture Department of University of Oradea, Romania.

A **B** **C**

Figure 1.A. *Hibiscus sabdariffa* (Red) **B.** *Hibiscus syriacus* (Oiseau Bleu or Blue Bird, Blue) **C.** *Hibiscus syriacus* (Gold Dust ,Yellow)

Preparation of Hibiscus extracts

For the quantification of total anthocyanins by spectrophotometer method, the air-dried powdered petals of *Hibiscus* were mixed with methanol and methanol acidulated with hydrochloric acid (0.1%) in the ratio 1:10 (w/v). The mixture was centrifuged at 15000 rpm for 20 minutes, and supernatants were used for analyses.

UV-Vis spectroscopy fingerprint of Hibiscus extracts

UV-VIS spectroscopy fingerprints of *Hibiscus* extracts were performed between 250 and 950 nm, after a prior dilution of extracts (1:5 v/v), using a Shimadzu UV-VIS 1240 mini, Shimadzu Corp. Kyoto, Japan spectrophotometer.

The total anthocyanins pigment content

The total monomeric anthocyanin pigments (MAP) content was measured using pH-differential method according to colour variation in function of pH as described by Giusti & Wrolstad (2001). *Hibiscus* samples were diluted with the buffers at pH 1.0 (0.025 M potassium chloride buffer) and pH 4.5 (0.4 M sodium acetate buffer). The samples were allowed to equilibrate for 15 minutes before the absorbance at λ=520 and 700 nm for each samples were recorded using Shimatzu mini UV-Vis spectrophotometer, calibrated with distilled water as the blank. The result was calculated as mg of cyanidin-3-glucoside/kg using the following equations:

$$\mathbf{A = (A_{\lambda 520} - A_{700})\ pH\ 1.0 - (A_{\lambda 520} - A_{700})\ pH\ 4.5}$$

$$\mathbf{Monomeric\ anthocyanin\ pigment\ (MAP)\ (mg/100g) = (A \times MW \times DF \times 20) / (\varepsilon \times 1) * m}$$

where the A is the absorbance calculated by equation 1, ε is the cyanidin-3-glucoside molar absorptivity (*26900* L.cm-1.mol-1), MW is the molecular weight for cyanidin-3-glucoside (*449.2*g/mol), m is the weight of sample and DF is a dilution factor.

Statistical analysis

All values are expressed as mean ± S.D. Data were subjected to one-way analysis of variance (ANOVA) and comparison among means was determined according to Tukey's test, significant differences were accepted at $P < 0.05$. The statistical tests were generated with GraphPad Prism version 5.00 for Windows.

RESULTS AND DISCUSSION

Ultraviolet and visible spectroscopy was one of the earliest techniques used for bioactive compounds analysis. Before starting the quantification of total anthocyanins pigments content an UV-Vis spectra fingerprint (250-950 nm) of methanol extracts from *Hibiscus* species was recorded in order to establish the maximum absorbtion, specifically bioactive compounds, of extracts. The fingerprint UV-Vis absorbtion spectra of three different *Hibiscus* species are shown in **Figure 2**.

The fingerprint spectra of all extracts showed characteristic peaks of phenolic acids (280 nm) and flavonoids. Flavonoids have two characteristic UV/Vis bands, band I in the 300 to 550 nm range, arising from the B ring, and band II in the 240 to 285 nm range, arising from the A ring (Pinheiro & Justino, 2012). Only in the case of HIB-blue and HIB_red were recorded the maximum absorbtion in the area 500-550 nm that are characteristic for anthocyanins pigments.

In order to quantification of total anthocyanins pigments content from *Hibiscus* samples, the methanol and methanol acidulated with hydrochloric acid were used as solvent and the results are shown in Table 1.

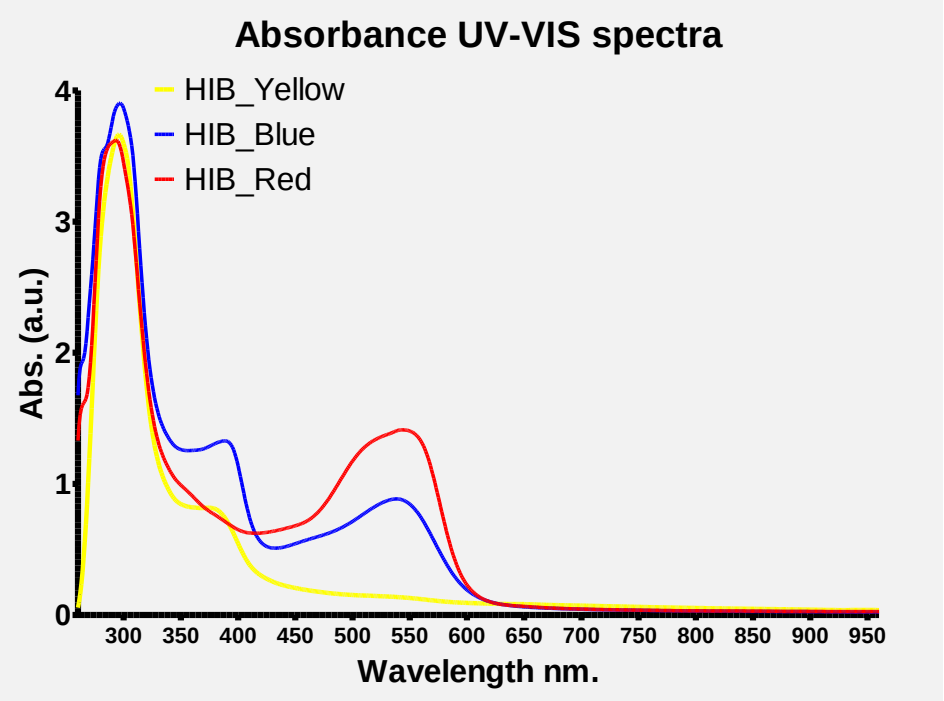

Figure 2. The UV-Vis spectra of three different Hibiscus species *(Hibiscus syriacus*, Gold Dust –HIB-yellow; *Hibiscus syriacus*, Oiseau Bleu – HIB_Blue; *Hibiscus sabdariffa* - *HIB_Red)*

Tabel 1

The total anthocyanins content (means ± SD) from two different species of *Hibiscus*

***Hibiscus* Samples**	**MAP (cyanidin-3-glucoside, mg/100g)**
HIB_Red	213.64±7.14[a]
HIB_Blue	37.29±3.33[b]
HIB_Red/HCL	361.37±5.32[c]
HIB_Blue/HCL	43.25±1.87[b,d]

Mean values are followed by different letters if are significantly different, according to Tukey's test. ($P<0.05$).

The highest content of anthocyanins was recorded in the case of *Hibiscus sabdariffa* (HIB_Red) comparative with the *Hibiscus syriacus* (HIB_Blue). The best extraction of anthocyanins were recorded when

acidulated methanol was used as solvent and the results are presented in the Figure 3.

Aishah et al., 2013 investigated the effect of pH on the content of monomeric anthocyanins in *H. sabdariffa*, *M. malabathricum* and *I. batatas*. Among the samples, *H. sabdariffa* contained the highest monomeric anthocyanins (163.32 ± 8.17 mg/L). The anthocyanins content of our *H. sabdariffa* extracts expressed as mg cyaniding-3-glucoside/L was only the 48.09 mg/L, results due to different method of pigment extraction. Bhaskar et al., 2011 made a phytochemical screening and evaluated in vitro antioxidant capacity of *Hibiscus rosa sinensis* L, and found that this plant could be a potential source of natural antioxidant and can be used as therapeutic agent.

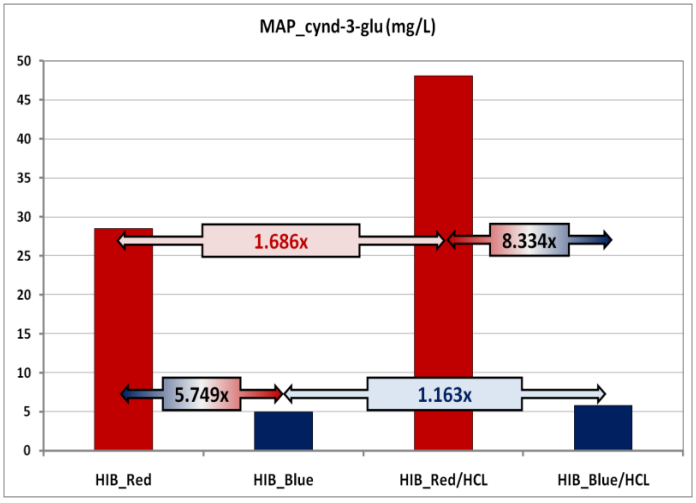

Figure 3. Total Anthocyanins Content (mg/L) of *Hibiscus* Species expressed as monomeric anthocyanin pigment and the difference (expressed as ratio between the higest to lowest anthocyanins content) between species and solvent used

Our previous work (Lestyan et al., 2014) two species of *Hibiscus* were investigated regarding to the *in vitro* antioxidant activity. The results shown that *H. sabdariffa* has the highest capacity comparative with *H. syriacus* probably due to the presence of anthocyanins.

CONCLUSIONS

The phytochemicals identified in the flower of *Hibiscus* species made it a potential source of natural antioxidant that could have a great importance in medicine and pharmacology. In this study we are focusing on the

anthocyanins content from three different species of *Hibiscus*, one of them, *H. sabdariffa* has the highest content of these bioactive compounds.

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