

**THE QUANTITATIVE ANALYSIS THROUGH  
SPECTROPHOTOMETRY OF FLAVONOIDS AND  
POLYPHENOLS FROM VEGETABLE PRODUCTS *HIBISCI  
TRIONI HERBA, RADIX AND FRUCTUS***

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**Abstract**

*This work presents the spectrophotometric determination of flavonoids and polyphenols from vegetable products Hibisci Trioni herba, radix and fructus. The determinations were done on methanolic extract of herba taken in 2011 and dried herba, roots and fruits taken in 2010. The quantitative spectrophotometric analysis was performed according to the officinal method. The content of flavonoids for the analyzed samples was expressed in rutozide and the polyphenol content of the analyzed samples was expressed as caffeic acid. The herba contains the most abundant polyphenols 0.98 g% (expressed as caffeic acid). Concentrations determined in herba for 2010 are higher than those for 2011. There is a higher concentration of polyphenols and flavonoids in the airy parts than in fruit.*

**Key words:** Hibisci Trioni Herba, flavonoids, polyphenol, spectrophotometry

**INTRODUCTION**

Medicinal plants have been a readily available source of drugs since ancient times and even today almost 50% of the new drugs have been patterned after phytochemicals (Saleem, et al., 2008). Recognizing the medicinal significance of indigenous plants, World Health Organization (WHO), in its 1997 guideline, states that “effective locally available plants can be used as substitutes for drugs” (Govindarajan, et al, 2003).

Plants have almost limitless ability to synthesize aromatic substances, most of which are phenols or flavonoids (Geissman, 1963; Papuc, et al., 2008; Papuc, et. Al. 2008). Polyphenols and flavonoids exhibit several biological effects such as anti-inflammatory, anti-microbial, anti-carcinogenic, anti-HIV, cardio-protective and neuro-protective.

Lately numerous studies have been conducted on the antioxidant effect of flavonoids (Dumitrescu, et al. 2009) and their content in different plants, fruits and vegetables (Palaghicius, et al. 2009; Condrat, et al. 2009) and polyphenols (Popescu, et al. 2011; Lungu et al. 2010; Papuc, et al. 2010).

*Hibiscus Trionum L.* (Zămoșița) species of the family *Malvaceae*, widespread in spontaneous flora of Romania is mentioned in the literature as

the descriptive botanical information: area of distribution, species description, its variability and empirical uses, known in traditional medicine (Silva, 1987). Farmacognostically speaking, the plant was studied (Rácz, Rácz-Kotilla, 1965; Szabo, et al. 2006).

*Hibiscus Trionum* is considered by most biologists and farmers generally just a weed". Wheat and corn are invaded by this undesirable species (Ceașescu, et al. 1984). It produces the most damage in dry years, being extremely resistant, it competes the other crops especially potato and maize (Ianoși, 2002).

Genus *Hibiscus* is best known for ornamental species (Beffa Della, 2003; Petulengró, 1994), and the species *Hibiscus Esculentus* (Okra) appreciated as a food in many countries in the Balkans and the Middle East (Bonnier, du Sablon, 1919; Jussieu, 1759). Okra is grown mainly in countries with warm climates, the green fruit called "gombo" being edible.

In the Romanian flora the *Hibiscus* genus is represented by *Hibiscus trionum* L. as native species with four subspecies: *H. Longilobus*, *H. Prostratus*, *H. Cordifolius*, *H. Ternatus*. *Hibiscus Trionum* L. - Zămoșița is the only species with known therapeutic use (\*\*\*, 1979; \*\*\*,1993).

Frequent in the plains and hills, in fields, gardens, ruderal places, dry to moist rich soil (Butura, 1979).

The plant product used is *Hibiscus Ttrionii herba* harvested in early flowering. The literature mentions therapeutic use and underground organ *Hibisci radix* (Cav. Cavanilles A. J., 1791–1801; Bojor, Alexan, 1981).

*Hibiscus Trionum* appears in different blends of tea, but so far we know only a few standard pharmaceutical products derived from plants (Bojor, Alexan, 1981).

Romanian products are used in Diuretic tea preparations no.2 and no. 3 and between all conditioned abroad stands a mixture of herbs which has included in the formula the *Hibiscus Trionum* species, produced by Kneipp.

## MATERIALS AND METHODS

The plant material is the herba, root and fruit of *Hibiscus Trionum* L., harvested from Oradea in 2010 respectively 2011. The fresh vegetable products were dried at room temperature, then were crushed and pulverized to fine powder size. The phytochemical analysis was carried out on ethanolic extracts obtained from the conditioned product.

### Preparation of extracts

Methanolic extract of *herba* harvested in 2010 (sample A):

10 g herba shredded at the sieve I is extracted with 50 mL methanol with stirring, for 15 minutes, then separated at about 1000 rotations / minute. The extract is filtered and brought to 50 mL with methanol.

Methanolic extract of *herba* harvested in 2011 (sample B):

10 g *herba* shredded at the sieve I is extracted with 50 mL methanol with stirring for 15 minutes then separated at about 1000 rotations / minute. The extract is filtered and brought to 50 mL with methanol.

Methanolic extract of roots harvested in 2010 (sample C):

5 g of shredded root in sieve II is extracted with 25 mL methanol with stirring for 15 minutes then separated at about 1000 rotations / minute. The extract is filtered and brought to 25 mL with methanol.

Methanolic extract of the fruit harvested in 2010 (sample D):

10 g chopped fruit in sieve IV are extracted with 50 mL methanol with stirring for 15 minutes then separated at about 1000 rotations / minute. The extract is filtered and brought to 50 mL with methanol.

The sample solutions were obtained by adding 1 mL methanolic extract of 5 mL sodium acetate (Merck) 100 g/L and 3 mL of aluminum chloride (Fluka) 25 g/L, filled to 25 mL with methanol and homogenized

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## RESULTS AND DISCUSSION

The quantitative analysis of flavonoids was performed according to the officinal method ((\*\*\*, 1979), the result being expressed in rutozide, % g/g After the first measurement, solutions were diluted 1:50 relative to the results, to be included on the scale of values. The absorbance of sample solutions was determined at a wavelength of 340 nm. The fluid for offset methanolic extract was prepared from 1 mL methanolic extract, 8 mL water, filled to 25 mL with methanol and homogenized. The apparatus used: Jasco spectrophotometer.

The calculation of concentration in flavonoids was performed by using a calibration curve, made by processing solutions rutozide (Roth) of known concentration under identical samples. Figure 3 shows the calibration curve and Table 1 shows the content of flavonoids expressed in rutozide for the analyzed samples.

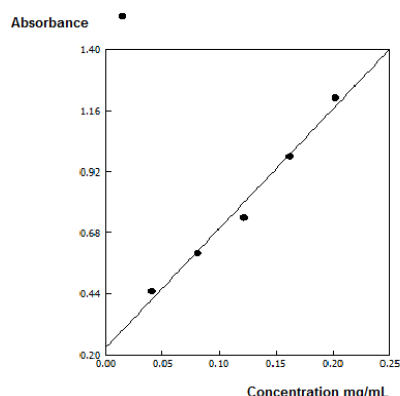


Fig. 3. The calibration curve in rutozide

The equation of the calibration line is:

Absorbance = 0,22 + 4,71 x concentration, and the correlation factor is  $r = 0,992$ .

Tabel 1

The flavonoids content expressed in rutozide

Sample	Absorbance			Medium absorbance	Concentration of total flavonoids, expressed in rutozide, %
	Determination 1	Determination 2	Determination 3		
A	0,996	0,993	0,996	0,995	2,06±0,025
B	0,932	0,928	0,928	0,929	1,88±0,025
D	0,592	0,592	0,593	0,592	0,99±0,007

The quantitative determination results presented in Table 1 show a significant variation depending on the concentration of flavonoids extracted from different plant organs and crop years. The quantitative determination of flavonoids in plant products analyzed the fact that methanolic extract of herba harvested in 2010 (sample C) contains the highest percentage of flavonoids 2.06 g% (as rutozide).

#### **The quantitative spectrophotometric analysis of total polyphenols**

The quantitative analysis of total polyphenols was achieved by spectrophotometric method, the total polyphenols expressed as caffeic acid. The quantitative determination of total polyphenols was done after the technique described in the monography *Cynarae folium*, by a spectrophotometric method based on color reaction time of these compounds with Arnow reagent (R) (\*\*\*,1993).

0.5 mL of methanol extracts is diluted with alcohol 50% vol to 50 mL in a volumetric flask. To 1 mL add 1 mL of dilute HCl (Merck) 0.5 N, 1 mL reagent Arnow, 1 mL sodium hydroxide (Fluka) and N make up to 10 mL by adding purified water in portions and stirring continuously. After 10 minutes read absorbance at a wavelength solution  $\lambda = 500$  nm in 1 cm cuvette, using as evidence in a white mixture of 0.1 mL undiluted extraction, 1 mL 0.5 N hydrochloric acid, 1 mL N sodium hydroxide in 10 mL supplemented by adding purified water. The polyphenol content of the analyzed samples was determined using a calibration curve built on values corresponding to absorbances of solutions at different concentrations of the caffeic acid (Roth). Measurements were performed with Jasco spectrophotometer. Figure 4 presents the calibration curve in caffeic acid, the equation of the curve and the correlation coefficient, and Table 2 presents the polyphenol content of the analyzed samples expressed as caffeic acid.

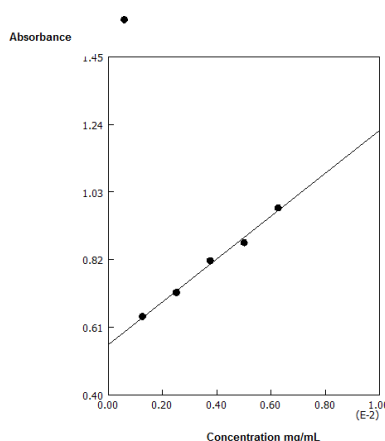


Fig. 4. The calibration curve in caffeic acid

The equation of the calibration line: Absorbance =  $0,56 + 66,32 \times \text{concentration}$ , and the correlation factor  $r$  is 0,996.

*Table 2*

The polyphenol content expressed in caffeic acid

Sample	Absorbance			Medium absorbance	Concentration of total polyphenol expressed in caffeic acid, %, g/g
	Determination 1	Determination 2	Determination 3		
A	0,601	0,600	0,601	0,601	$0,98 \pm 0,074$
B	0,591	0,590	0,590	0,590	$0,73 \pm 0,074$
C	0,640	0,641	0,641	0,641	$0,012 \pm 0,0002$
D	0,584	0,582	0,584	0,583	$0,56 \pm 0,124$

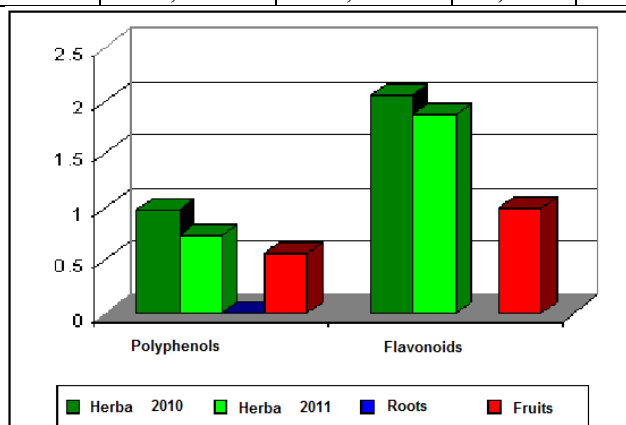
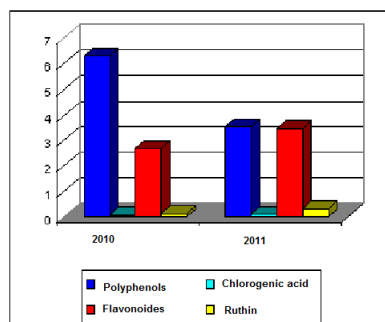


Fig. 5. The evolution of the concentration in total polyphenols and total flavonoids



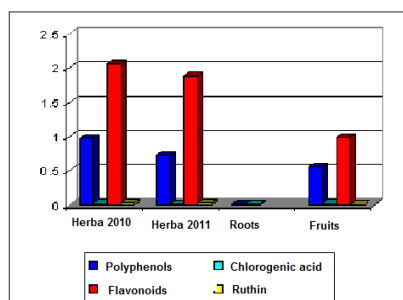


Fig. 6. The variation of concentrations in different parts of the plant and in time

The chlorogenic acid is an important representative of the class of phenylcarboxylic acids present in the plant studied and is a representative class rutoside flavonoids present in plants.

The histograms presented in Fig. 5 and 6 present the evolution of the concentration of total flavonoids, total polyphenols and chlorogenic acid. The evolution refers to different plant parts for the same vegetable, but harvested in different years. It can be observed that is a higher concentration of polyphenols and flavonoids in the airy parts than in fruit.

## CONCLUSIONS

After analyzing the results, it was found that herba contains the most abundant polyphenols 0.98 g% (expressed as caffeic acid).

Concentrations determined in herba for 2010 are higher than those for 2011; the difference is greater than that determined for flavonoids 0.25 to 0.18 g/g %

There is a higher concentration of polyphenols and flavonoids in the airy parts than in fruit.

The vegetable products harvested in 2010 had the content in polyphenols and flavonoids higher than in 2011.

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