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HPLC DETERMINATION OF POLYPHENOLS FROM *CALENDULA OFFICINALIS* L. FLOWERS

– Short communication –

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Abstract: Romanian spontaneous flora provides a lot of resources for the determination of different chemical compounds. This study uses flower samples from *Calendula officinalis* L. extracted through maceration. The chemical compounds determined were: (+)- catechin, caffeic acid, chlorogenic acid, cinnamic acid, ferulic acid, gallic acid, rutin, resveratrol and quercetin. They were analyzed by using an optimized HPLC method. (+)-Catechin, caffeic acid, chlorogenic acid and quercetin could not be identified in the analyzed samples. The greatest amount of phenolic compound found was rutin and the smallest quantity was determined for ferulic acid. The quantified compounds have proven to have benefits regarding human health, thus they can be used as functional compounds and can be included in food products and food supplements.

Key words: common marigold, functional compounds, HPLC, phenolic compounds

INTRODUCTION

Chemical compounds extracted from plants have been used for a long period of time for the prevention of diseases or as adjuvants in their treatment. Several properties as antifungal (Mironescu et al., 2009, Georgescu & Mironescu, 2011), antibacterial and antiviral have been proved to be useful in the preservation of human health (Swamy et al., 2016).

Phenolic compounds are of great interest nowadays due to their benefits regarding human health. They can be used as adjuvants in the treatment of several diseases, mostly oxidative-stress related diseases, because of their antioxidant properties (Martins et al., 2016, Zhang et al., 2016). Thus they can be used in cardiovascular, neurodegenerative and gastrointestinal diseases, cancer (Martins et al., 2016, Aguilera et al., 2016), diabetes (Liu et al., 2016), obesity (Hernandez-Saavedra et al., 2015) and inflammatory diseases (Petrova et al., 2016).

Calendula officinalis L. is a plant that grows annually and that is included in the A*steraceae* family (El-Nashar et al., 2016). It has a flower stem that can reach 60 cm and the color of the flowers can vary from yellow to orange. They bloom starting from June to late autumn (Sausserde & Kampus, 2014). It is commonly found in Romaniain the spontaneous flora as well as in the cultivated one.

Its' chemical composition depends on the region and the period in which the plant has been harvested (Gomez Honorio et al., 2016). Thus the diversity in chemical compounds that was determined lead us to examine the content in several phenolic compounds that poses health benefits from the plants harvested from Romania.

Studies regarding the uses of *Calendula officinalis* L. flowers in food industry show that they can be consumed fresh (di Tizio et al., 2012), dried or processed as tea, candy or liqueur (Acikgoz, 2017).

The aim of this study is to analyze the *Calendula officinalis* L. flowers regarding their content in several chemical compounds that have been proven to possess health benefits.

MATERIALS AND METHODS

Extraction

The flowers of *C. officinalis* L. were harvested from Romania, Sibiu County in august, when the flowers reached maturity. The extract was made by maceration of 1g of dried flowers in 10 mL of purified water for 72 hours at room temperature. After the time expired the sample was filtered and analyzed using an HPLC method.

Analysis

The determination of the analyzed phenolic compounds regarding their identity and quantity was carried out by using an HPLC system, 1200 series provided by Agilent Technologies. The column that was used in order to complete the analysis was the Zorbax Eclipse Plus C18 with the following dimensions: 250 mm x 4,6 mm i.d. x 5µm at 25ºC. The elution was chosen by using three mobile phases. Mobile phase (m.p.) A was purified water, B, methanol and C,purified water: glacial acetic acid (96:4). The method followed a strict gradient program: at 0 min 15% m.p.B and 85%m.p. C, at 15 min,75% m.p. A and 25% m.p. B, at 20 min, 15% m.p. A and 85% m.p. B, at 40 min, 40% m.p. A and 60% m.p. B, at 45 min, 5% m.p. A and 95% m.p. B, at 55 min, 5% m.p. A and 95% m.p. B, at 60 min, 85% m.p. A and 15% m.p. B and at 70 min, 85% m.p. A and 15% m.p. B. The used flow rate was gradient style too. At 0 min the flow rate was 0.5 mL/min and from 15 to 70 min 0.8 mL/min. 5 µL was the injection volume used and the wavelengths were 360, 330, 303 and 280 nm (Frum et al., 2017). The standard compounds used were of HPLC purity and came from Sigma Aldrich.

The quantities of the phenolic compounds in the analyzed samples were determined depending on the compounds' aria from the standard chromatogram. In order to acquire an exact quantification of the phenolic compounds, the analysis was performed in triplicate.

RESULTS AND DISCUSSIONS

The qualitative determination of the chemical compounds analyzed was accomplished by the comparison of the retention times (R_T) of the phenolic compounds determined in the standard mixture chromatogram (Figure 1) to the compounds found in the sample chromatogram (Figures 2 and 3) at each compounds' specific wavelength. The (+)-catechin, cinnamic acid, gallic acid and syringic acid were identified at 280 nm, resveratrol at 303 nm, ferulic acid, caffeic acid and chlorogenic acid at 330 nm and quercetin and rutin at 360 nm. Thus several compounds, like quercetin, chlorogenic acid, caffeic acid and (+)- catechin, were not detected. (Table 1, Figures 1-3).

Table 1. The identification of phenolic compounds

Wavelength	Compound	Standard R_T (min)	Sample R_T (min)
280 nm	Gallic acid	6.68	6.69
	(+)-Catechin	12.51	-
	Syringic acid	20.57	20.51
	Cinnamic acid	24.37	24.42
303 nm	Resveratrol	22.82	22.87
330 nm	Chlorogenic acid	15.46	-
	Caffeic acid	20.30	-
	Ferulic acid	22.37	22.35
360 nm	Rutin	22.51	22.47
	Quercetin	23.76	-

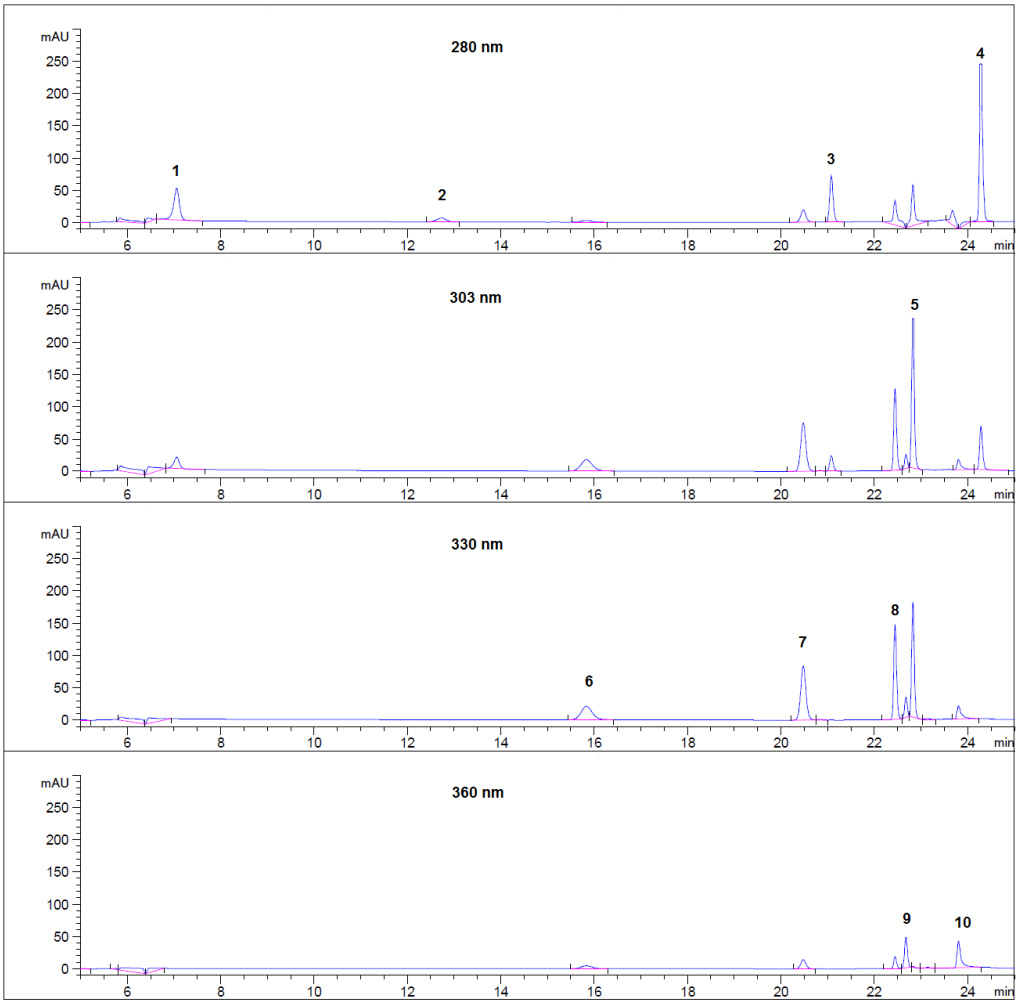

Figure 1. Graphic representation of the mixture of standards
1. Gallic acid, 2. (+)-Catechin, 3. Syringic acid, 4. Cinnamic acid, 5. Resveratrol, 6. Chlorogenic acid, 7. Caffeic acid, 8. Ferulic acid, 9. Rutin, 10. Quercetin

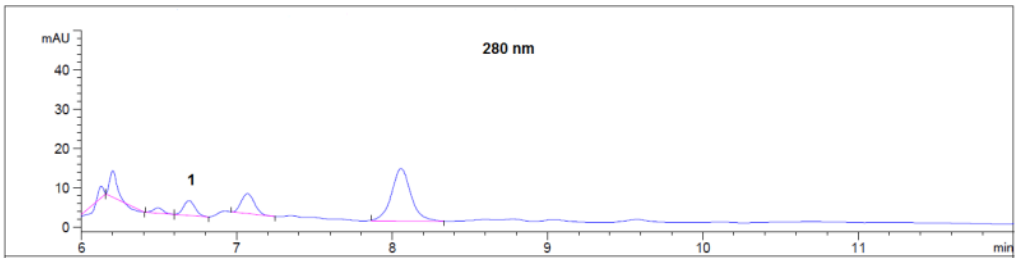

Figure 2. Graphic representation of the analyzed sample
1. Gallic acid

The greatest quantity of phenolic compound quantified was 3.10 mg/ 100 g vegetal product (v.p.) for rutin, followed by 2.79 mg / 100 g v.p. for syringic acid. Quantities below 1 mg / 100 g v.p. were determined for cinnamic acid: 0.83 mg / 100 g v.p., gallic acid: 0.8 mg / 100 g v.p., resveratrol: 0.49 mg / 100 g v.p., and the smallest quantity: 0.24 mg / 100 g v.p. was determined for ferulic acid (Fig. 4).

The determined phenolic compounds posses a great interest regarding human health, thus they can be used in the food industry like functional compounds.

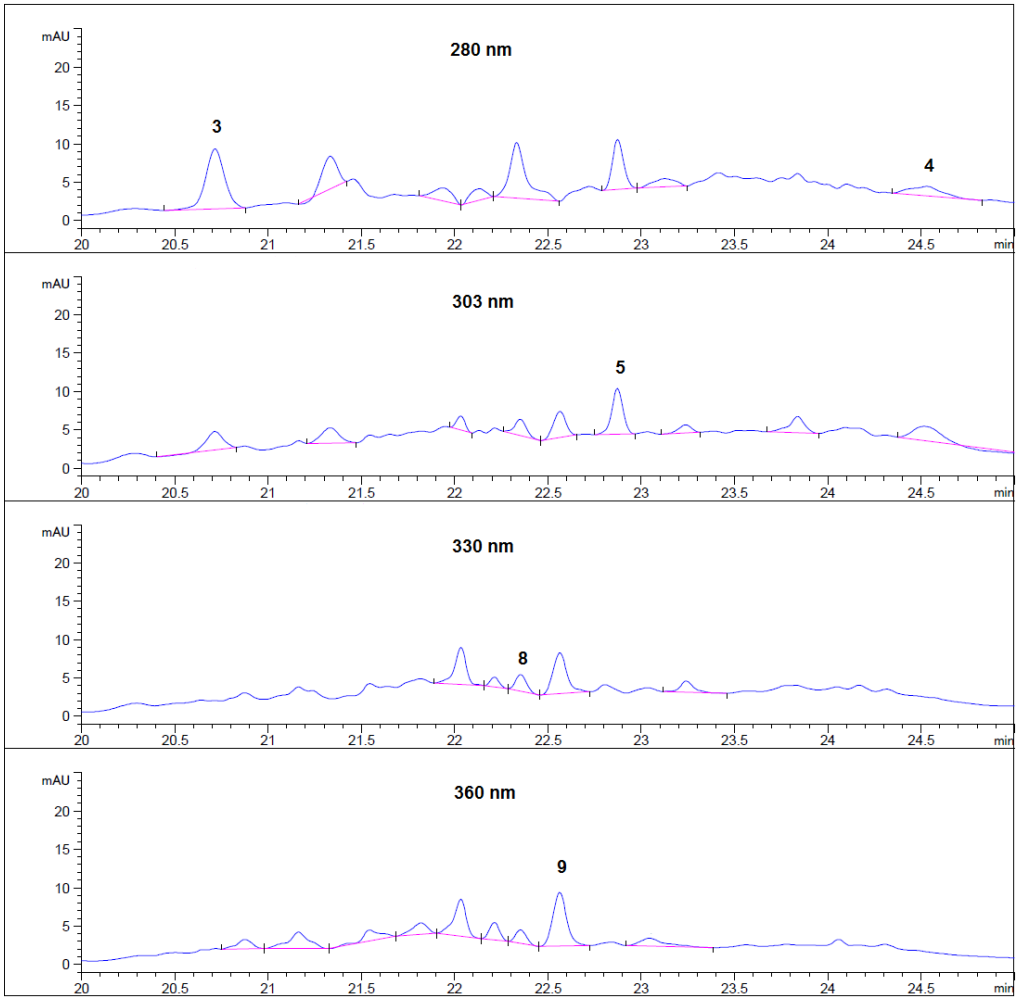

Figure 3. Graphic representation of the analyzed sample
3. Syringic acid, 4. Cinnamic acid, 5. Resveratrol, 8. Ferulic acid, 9. Rutin

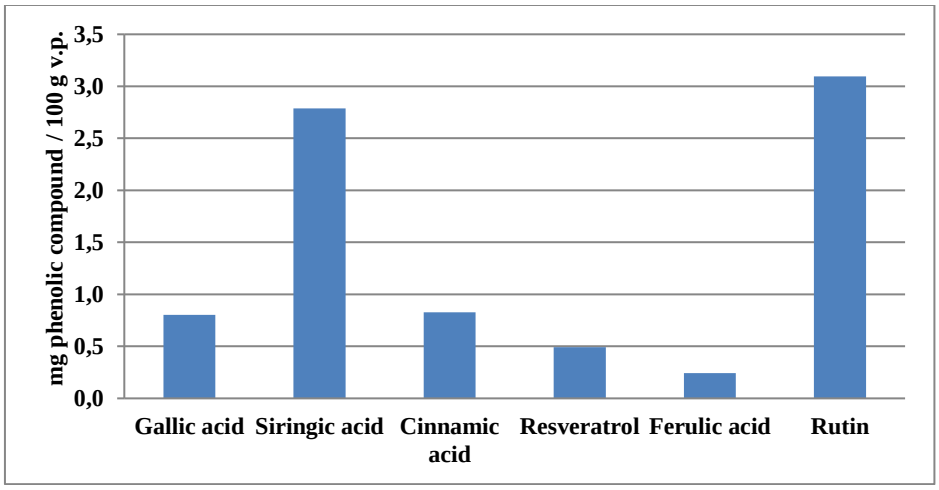

Figure 4. The quantification of several phenolic compounds

CONCLUSIONS

The quantities of chemical compounds in vegetal products can fluctuate depending on the geographical region of growth, time of harvesting, climate and soil composition.

This study was based on the qualitative and quantitative determination of several chemical compounds extracted from flowers of a common plant from Romania. Several compounds, like (+)- catechin, chlorogenic

acid, caffeic acid and quercetin were not detected.

The greatest quantity of phenolic compound was determined for rutin and the lowest for ferulic acid.

Due to the phenolic composition of the *C. officinalis* L. flower extract, it can be used in the industry as ingredients for several types of food for the obtaining of food with health benefits or dietary supplements.

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