PERIODICO di MINERALOGIA Volume 88, No. 6, 2019

Qualitative and Quantitative Determination of the Caffeic Acid and Chlorogenic Acid from Three Chemovarieties of *Chrysanthemum balsamita L*

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Abstract

This paper presents the studies performed on three chemovarieties of the species Chrysanthemum balsamita L for the identification and dosage of the phenyl propane derivatives such as caffeic acid and chlorogenic acid which confers to these species remarkable pharmaceutical properties (hepatoprotective, cholagog – choleretic). Using the techniques of thin layer chromatography, densitometry and the UV-VIS spectra we have found that the three chemovarieties of Chrysanthemum balsamita contain, in the herb plant, caffeic acid and chlorogenic acid ranging between 0.64 up to 1.61% and respectively 0.38 up to 0.4%.

Keywords: caffeic acid, quantitative and qualitative determination, *chrysanthenum balsamita*

Introduction

The *Chrysanthemum balsamita L* species (Asteraceae), costmary, is a herbaceous, perennial plant and it is well known for its chemotaxonomic variety. The active principle is the volatile oil contained by the flowers and leaves. The volatile oil differs from one chemovariety to another by its main chemical composition component.

Two chemical taxons are known in Romania: *Ch.balsamita chemovar carvona* (which contains about 60% carvona in the volatile oil) and *Ch.balsamita chemovar camphora* (which contains about 85% camphor in the volatile oil). The third chemical taxon brought from The Botanical Gardens of Kishinev has already been acclimatized at the Medicinal Plants Laboratory in Brasov [1,2]. If we are to take into account the main component present in the volatile oil of this chemovariety we can call it (using the similarity with the others) *Ch.balsamita chemovar. thujona* (which contains about 40% thujona).

Experimental

Chemicals and Materials

The herba of the *Chrysanthemum balsamita var. camfora* (Cb camf.); *Chrysanthemum balsamita var. carvona* (Cb carv.) and *Chrysanthemum balsamita var. thujona* (Cb thuj.) were obtained from the Medicinal Plants Research Laboratory Brasov.

The analytical purity methanol was used for extraction and as solvent for standard solutions. The caffeic acid was provided by Roth (Karlsruhe, Germany) and the chlorogenic acid by Fluka (Swiss).

The thin – layer chromatography was performed on precoated silicagel 60 F_{254} plates. The solvents used to prepare the mobile phase were ethyl acetate from Carlo Erba (Italy) and formic acid from Roth (Karlsruhe, Germany). The Neu-PEG reagent was used as spray reagent (1- % methanolic solution of diphenylborate of aminoethanol and 5 % methanolic solution of polyethylenglicol 400), Roth (Karlsruhe, Germany) supplied the reactives.

Apparatus

The thin – layer chromatography was performed with Desaga AS 30 automatic applicator, normal chromatographic chamber, and Reprostar II Camag apparatus.

The densitometry and the "in situ" UV-VIS spectra was performed with a Desaga CD 60 densitometer

Experimental conditions

- The TLC development:
- plate: silicagel 60 F_{254};
- samples: the methanolic extracts were made by maceration in 24 hour.
    a. methanolic extract from Cb camf. (1,0526 g herba in 10 ml methanol)
    b. methanolic extract from Cb carv. (0,9377 g herba in 10 ml methanol)
    c. methanolic extract from Cb thuj. (0,9859 g herba in 10 ml methanol)
- standards: caffeic acid 1,05 mg/ml in methanol
  chlorogenic acid 1,05 mg/ml in methanol
- development distance: 7 cm.
- mobile phase: ethyl acetate – formic acid – water (80 : 10 : 10, v/v).
- The densitograms were obtained at 254 nm in reflection.
- The UV–VIS spectra were performed "in situ" one the plate, between 200 and 700 nm.

Results and Discussions

The chromatograms of the samples were visualized in UV light at 254 nm. The plate was sprayed with Neu-PEG reagent and the chromatograms were visualized in UV light at 365 nm. The chromatograms of the samples show the presence of the spots with same color and at the same R_f values as the standards. **Figure 1** and **2** show the chromatograms of the samples and the standards at 254 nm and 365 nm sprayed with Neu-PEG reagent.

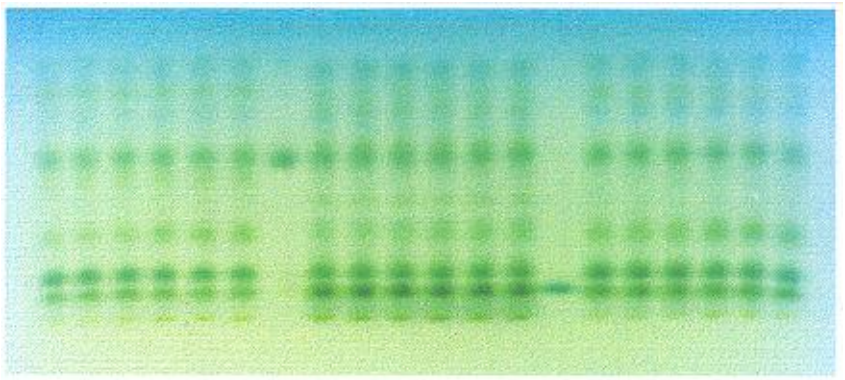


Figure 1. The chromatograms of the samples and the standards in UV light at 254 nm

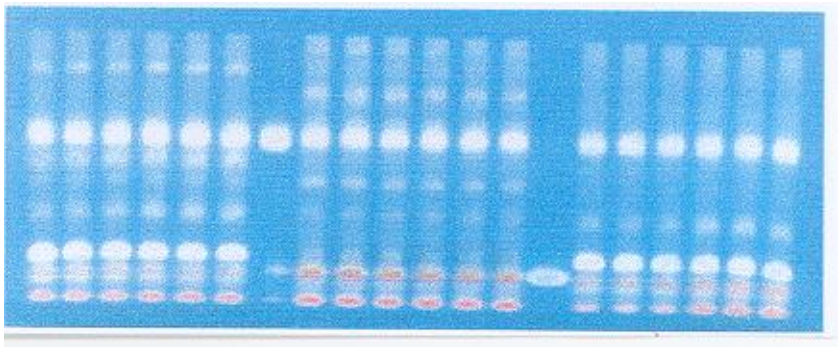
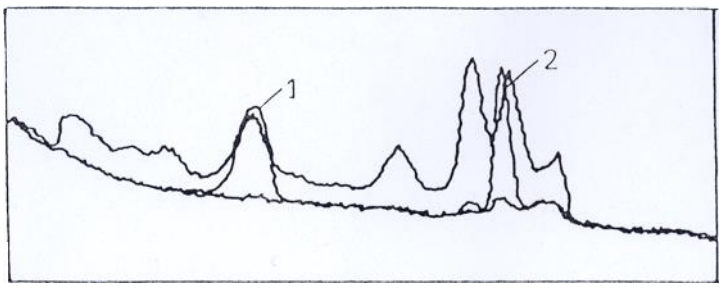


Figure 2. The chromatograms of the samples and the standards with Neu-PEG reagent, in UV light at 365 nm

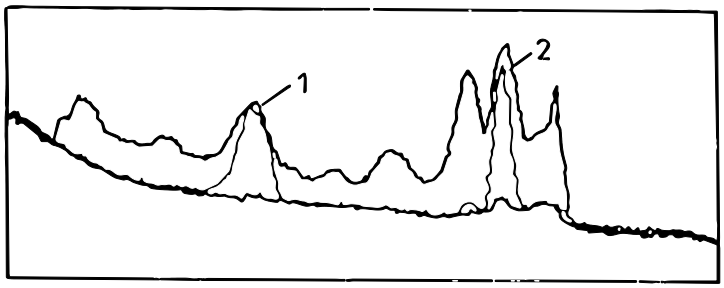
Figures 3-5 show the densitograms of the samples with the standards. It can be observed the presence of the peaks in the samples densitograms, at the same R_f values, as the peaks of the standards.

Figure 6 shows the densitograms of all three samples, which proved the presence of a few different components in these samples.



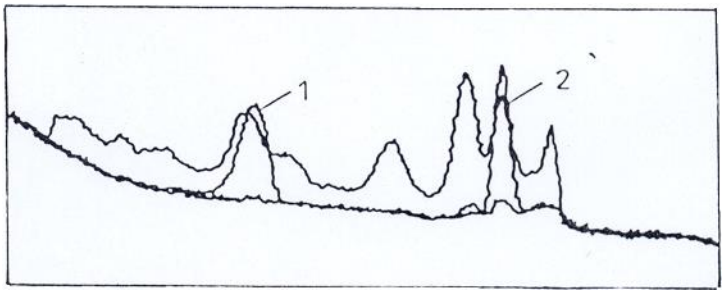
1- chlorogenic acid 2- caffeic acid

Figure 3. The densitograms of the *Cb. camf* and the standards at 254 nm



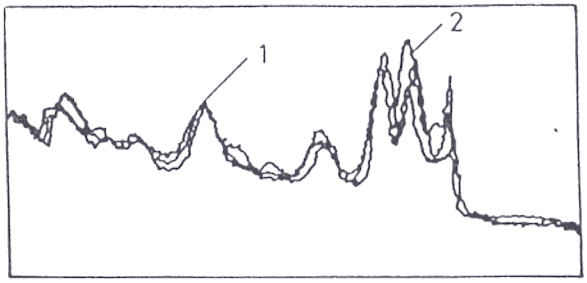
1- chlorogenic acid 2- caffeic acid

Figure 4. The densitograms of the *Cb.carv*. and the standards at 254 nm



1- chlorogenic acid 2- caffeic acid

Figure 5. The densitograms of the *Cb thuj.* and the standards at 254 nm



1- chlorogenic acid 2- caffeic acid

Figure 6. The densitograms of the three chemovarieties samples (*Cb. Camf., Cb.carv*., *Cb thuj.*)

The presence of the caffeic acid and the chlorogenic acid in the samples was proven by comparison of the UV-VIS spectra of the standards with the UV-VIS spectra of the separated components from the samples.

Figures 7-14 show the "in situ" UV-VIS spectra.

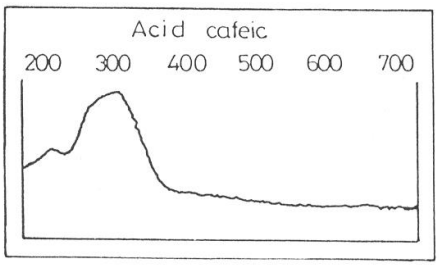


Figure 7. The UV-VIS spectra of the standard caffeic acid

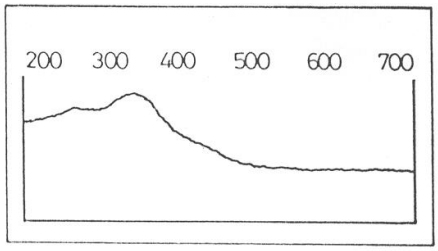


Figure 8. The UV-VIS spectra of the caffeic acid separated from Cb camf

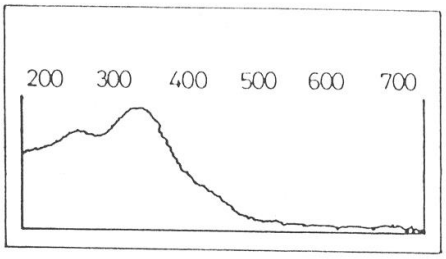


Figure 9. The UV-VIS spectra of the caffeic acid separated from Cb carv

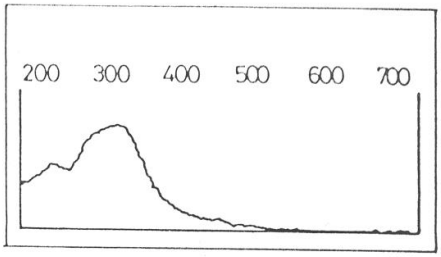


Figure 10. The UV-VIS spectra of the caffeic acid separated from Cb thuj

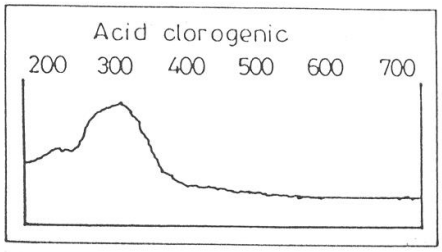


Figure 11. The UV-VIS spectra of the standard chlorogenic acid

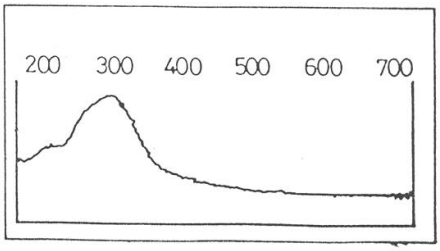


Figure 12. The UV-VIS spectra of the chlorogenic acid separated from Cb camf

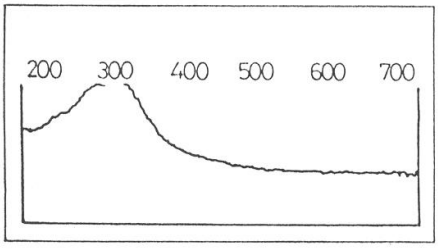


Figure 13. The UV-VIS spectra of the chlorogenic acid separated from Cb carv

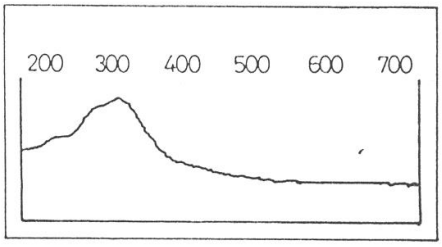


Figure 13. The UV-VIS spectra of the chlorogenic acid separated from Cb thuj

The calculated R_f values (**Table 1**) for the standards and the separated components from the samples demonstrates also the presence of the caffeic acid and the chlorogenic acid in this three chemovarieties of *Ch. balsamita L.*

Table 1. The calculated R_f for the standards and for the separated spots from samples

Samples	R_f for caffeic acid	R_f for chlorogenic acid
Standards	0,62	0,14
Cb camf.	0,62	0,14
Cb carv.	0,62	0,14
Cb thuj.	0,62	0,14

The quantitative determination was performed by TLC – densitometry using the calibration curve method.

Figures 15, 16 show the calibration curves obtained for the caffeic acid respectively the chlorogenic acid with photodensitometer Desaga CD 60. The calibration curves were performed by Slide Computer Program.

The equations of this curve are:

- for caffeic acid: $A = 30955,03+3794,88V$
- for chlorogenic acid: $A = 769,07+265,76V$

where A is the peak area and V is the applied volume in a spot.

The concentration was obtained with the formula:

$$C\%\ g/g = V_eC_{et}/20m$$

where C% (g/g) is the concentration; V_e is the coresponding volume from the standard, C_{et} is the concentration of the standard solution, 20 is the quantity of samples in μl, and m is the weight of the plant used for extraction.

Table 2. The concentration obtained from the caffeic acid and the chlorogenic acid from the Chrysanthemum balsamita chemovar camphora, Chrysanthemum balsamita chemovar. carvona and Chrysanthemum balsamita chemovar thujona is:

Samples	Caffeic acid %	Chlorogenic acid %
Ch.bals.camphora	0,97 % $\pm$ 0,23	0,38 % $\pm$ 0,03
Ch.bals.carvona	1,61 % $\pm$ 0,31	0,40 % $\pm$ 0,03
Ch.bals. thujona	0,64 % $\pm$ 0,16	0,38 % $\pm$ 0,01

It can be observed that the concentration of the caffeic acid is different in these three samples, but the concentration of the chlorogenic acid is nearly equal. The hepatoprotective and

cholagog-choleretic action of the species [5] is justified by the presence of the phenyl propanic derivatives.

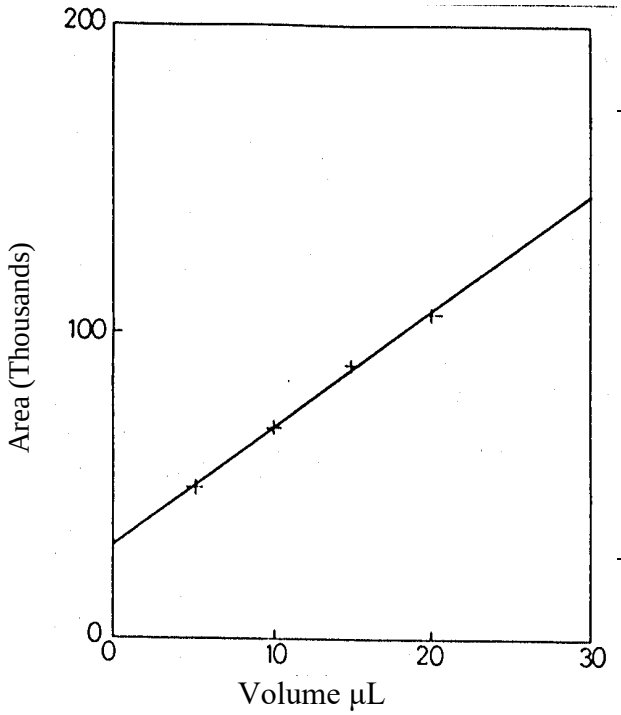


Figure 15 The calibration curve for caffeic acid

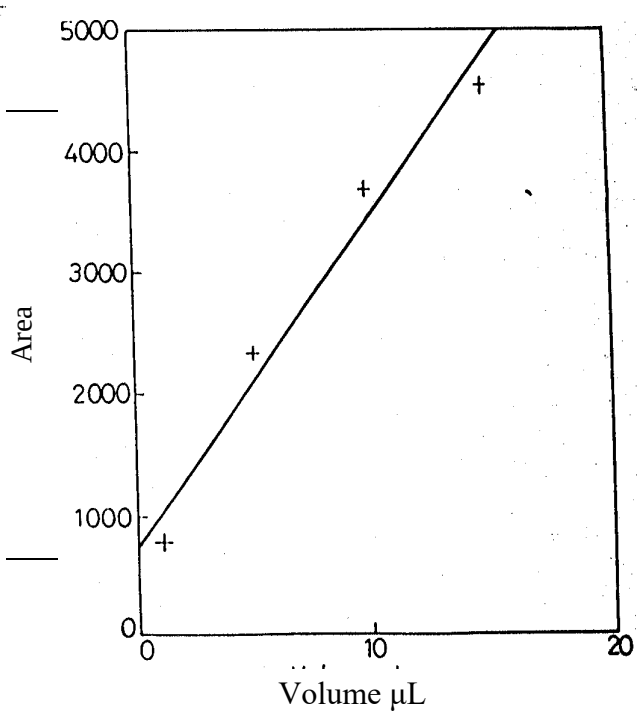


Figure 16 The calibration curve for chlorogenic acid

Conclusions

The caffeic acid and the chlorogenic acid were determined qualitatively and quantitatively in the three chemovarieties of *Chrysanthemum balsamita L.*, using chromatographic and spectral methods. The caffeic acid and the chlorogenic acid, responsible of the hepatoprotective and cholagog-choleretic action, are the most important phenyl propane derivatives components found in the chemovarieties of the *Ch. balsamita* species.

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