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Spectroanalytical Profile of Flavonoids from *Chelidonium majus L.*

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Abstract

The paper presents the spectroanalytical profile of the flavonoids from organs of Chelidonium majus L. (celandine). Firstly the extraction of flavonoids from the plant was done and then the separation of the heterosidic and aglyconic forms from the extracts. Then, the separation of the flavonoids from the heterosidic and aglyconic extracts was done. The identification and differentiation of the pure flavonoids and their chemical structure determination was achieved too. It was established that the stems, leaves and flowers of celandine contain a single flavonoid (similar for all those organs), which is a flavonol in the aglyconic form. The fruit and the seeds do not contain flavonoids.

Keywords: *Chelidonium majus L.*, celandine, flavonoids, analytical planar chromatography, circular technique, bidimensional technique, color tests, UV spectrometry, IR spectrometry.

Introduction

Chelidonium majus L. is a medicinal plant, used in folk medicine for its antitumoral, antimicrobial, antiviral, antimycotic, cholereric–cholagog, hepatoprotector properties [1]. The main active principles involved in those therapeutic activities are alkaloids, enzymes with antimytotic activity and flavonoids.

The spectroanalytical profile of alkaloids and enzymes from celandine is completely cleared up. This paper presents the spectroanalytical profile of those active principles, because the study of flavonoids from celandine is not completed.

In **(Figure 1)** we have presented the algorithm for the separation, partial purification, identification, differentiation and chemical structure determination of flavonoids from celandine.

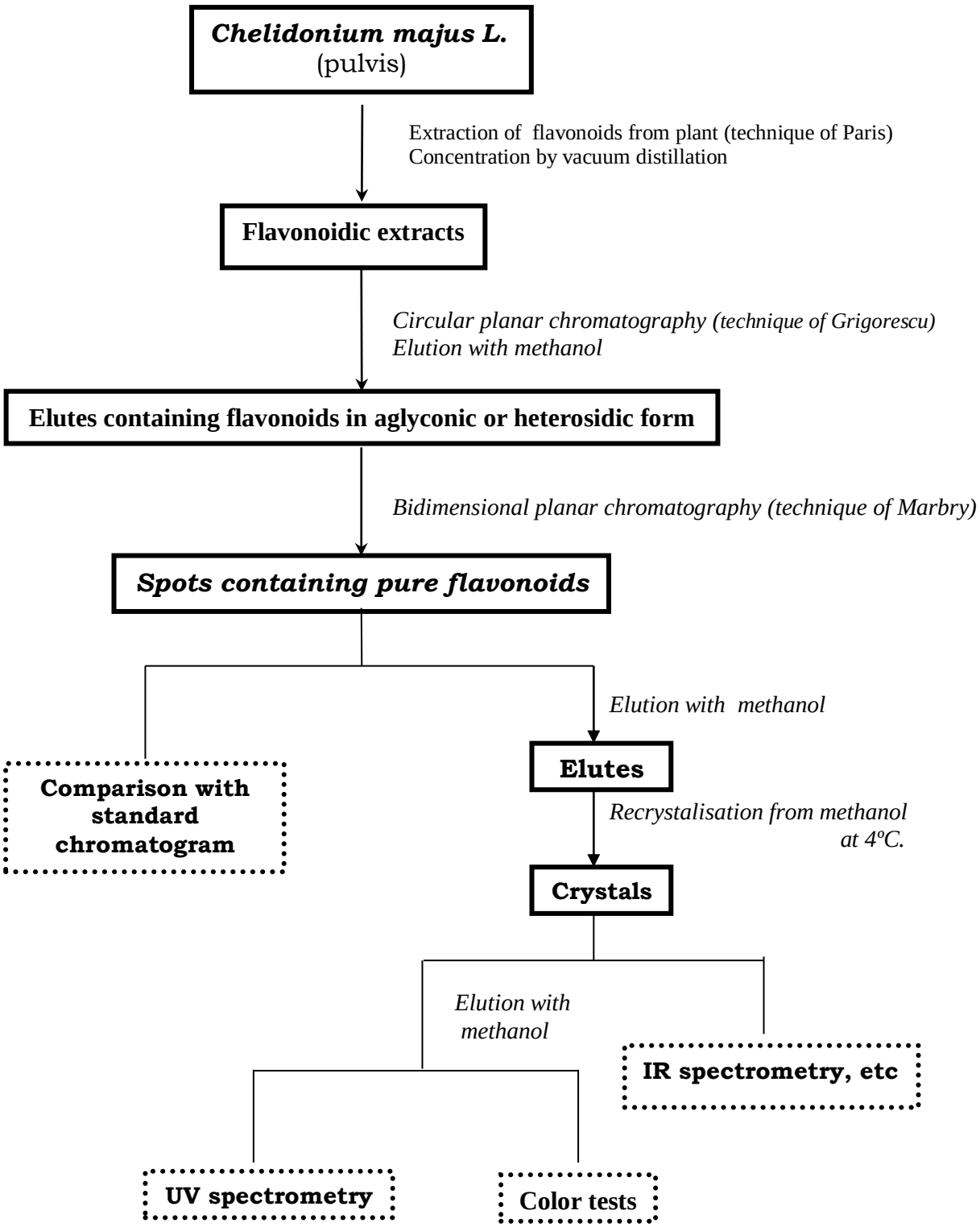


Figure 1. Procedure concerning extraction, purification, identification, differentiation and chemical structure determination of flavonoids from *Chelidonium majus.*

Materials and Methods

Materials

✓ Samples: fresh organs (steams, leaves, flowers, fruit and seeds) from Chelidonium majus L. harvested in June 2001, from Constantza;

✓ methanol p.a.; 5% $FeCl_3$; 5% NaOH; 5% $SbCl_3$; Mg pulvis, HCl p.a. (ρ=1,19), glacial acetic acid, isobutanol p.a., secured from the *Comchim Concern* (Romania);

✓ mobile phases:

- methanol p.a. : glacial acetic acid : distilled water (4 : 0.25 : 6 ; V:V:V);
- isobutanol p.a. : glacial acetic acid : distilled water (3 :1 : 1 ; V:V:V);
- glacial acetic acid : distilled water (3 :17 ; V:V);

✓ stationary phases:

- filter paper with medium pores (25 cm x 25 cm), secured from the *Whatman Concern* (England);
- Whatman no. 1 chromatographic paper (57 cm x 57 cm), secured from the *Whatman Concern* (England);

✓ UV-Vis spectrometer (*Camspek M 330*);

✓ IR spectrometer (*Vectra 2000 IR*).

Methods

- The extraction of the flavonoids was achieved using the technique of R. Paris [2]; the organs of celandine have been used as samples (except for the roots, which are ecologically protected).

Five extracts were obtained: one from stems, one from leaves, one from flowers, one from fruit and one from seeds.

After general color tests for the identification of flavonoids (Shibata test and reaction with 5% $FeCl_3$), it was established that only the extracts obtained from steams, leaves and flowers contained flavonoids (fruit and seeds did not contain that).

- The separation of heterosidic flavonoids from the aglyconic flavonoids from the extracts was done by analytical circular planar chromatography (the technique of E. Grigorescu) [3, 4]. The mixture methanol p.a. : glacial acetic acid : distilled water (4 : 0.25 : 6 ; V:V:V) was used as mobile phase and filter paper (25 cm x 25 cm) with medium pores as stationary phase.

Three analytical chromatograms were obtained, each one presenting two circular chromatographic bands: a brown internal one (containing alkaloids - Dragendorff test is positive) and a yellow external one (containing flavonoids - Shibata test is positive).

- The separation of flavonoids from each yellow band of the circular chromatograms. Each yellow band was eluted with 70% methanol; each elute was submitted to a bidimensional analytical chromatography (the technique of Marbry) [5], using Whatman paper no.1 as stationary phase, the mixture isobutanol p.a.: glacial acetic acid : distilled water (3:1:1; V:V:V) as mobile phase for the first migration, the mixture glacial acetic acid : distilled water (3:17 ; V:V) as mobile phase for the second migration. 26 hours were necessary for the first migration and 4 hours for the second migration

Three analytical chromatograms were obtained, each one containing a single spot with almost the same R_f (0,14).

- For the identification and differentiation of the separated flavonoids from the bidimensional chromatograms, the position of the spots were compared with the areas from the standard Marbry chromatogram [5] on which, for each group of flavonoids corresponds a characteristic area**.**

- For a better purification of the separated flavonoids, the spots were eluted (with methanol 70%) from the bidimensional chromatograms and then recrystalised by incubation of the elutes for 24 hours at 4°C, followed by centrifugation at 5000 rot/min for 15 min and drying at 50°C^0. The dried precipitates represented crystals of pure separated flavonoids.
- For a better identification and differentiation of the flavonoids, the crystals were solubilised in methanol 70% and submitted to color tests and UV spectrometry (using *a Camspek 200 UV-Vis* spectrometer).
- For the determination of the possible chemical structure of the separated flavonoids, the crystals were submitted to IR spectrometry (using a *Vectra 2000 IR* spectrometer). Three IR spectra were obtained.

Results and Discussions

Each experimental bidimensional chromatogram contained a single spot. The R_f was similar for all spots of the three chromatograms (R_f = 0,14).

It was concluded that the steams, leaves and flowers of celandine contain a single flavonoid, which is the same for all. In addition, comparing the position of the spots from the experimentally obtained chromatograms with the standard chromatogram given by Marbry (on which, a specific area corresponds for a specific group of flavonoids), it was concluded that the spots from the experimental chromatograms are flanonoids that belong to the specific area of flavonic, flavonolic, calconic or auronic aglycons.

The results of the tests based on color reactions (realized on the elutes of the bidimensional chromatograms) allow the conclusion that the flavonoidic aglycon is not a flavone, calchone or aurone (the specific reactions were negatives) – **(Table 1)**; this mean that the flavonoidic aglycon was a flavonol.

Table 1. Results of color tests for the identification of the flavonoidic compound separated from *Chelidonium majus L.*

Color reagent	Obtained color	Observations
NaOH	Yellow	The presence of the yellow color indicates that the separated compound has a flavonoidic structure.
$FeCl_3$	Green-blue	
$SbCl_3$	Yellow	The presence of the yellow color indicates that the separated compound has not a calchonic nature.
Mg+ HCl	Red	The presence of the red color indicates that the separated compound has a flavonolic nature.

From the three UV spectra **(Figure2)** of the three elutes of the spots from the bidimensional chromatograms it can be observed that all spectra had similar maximum of absorption**:**

λ_{max} = 245 nm ; λ_{max} = 275 nm ; λ_{max} = 345 nm.

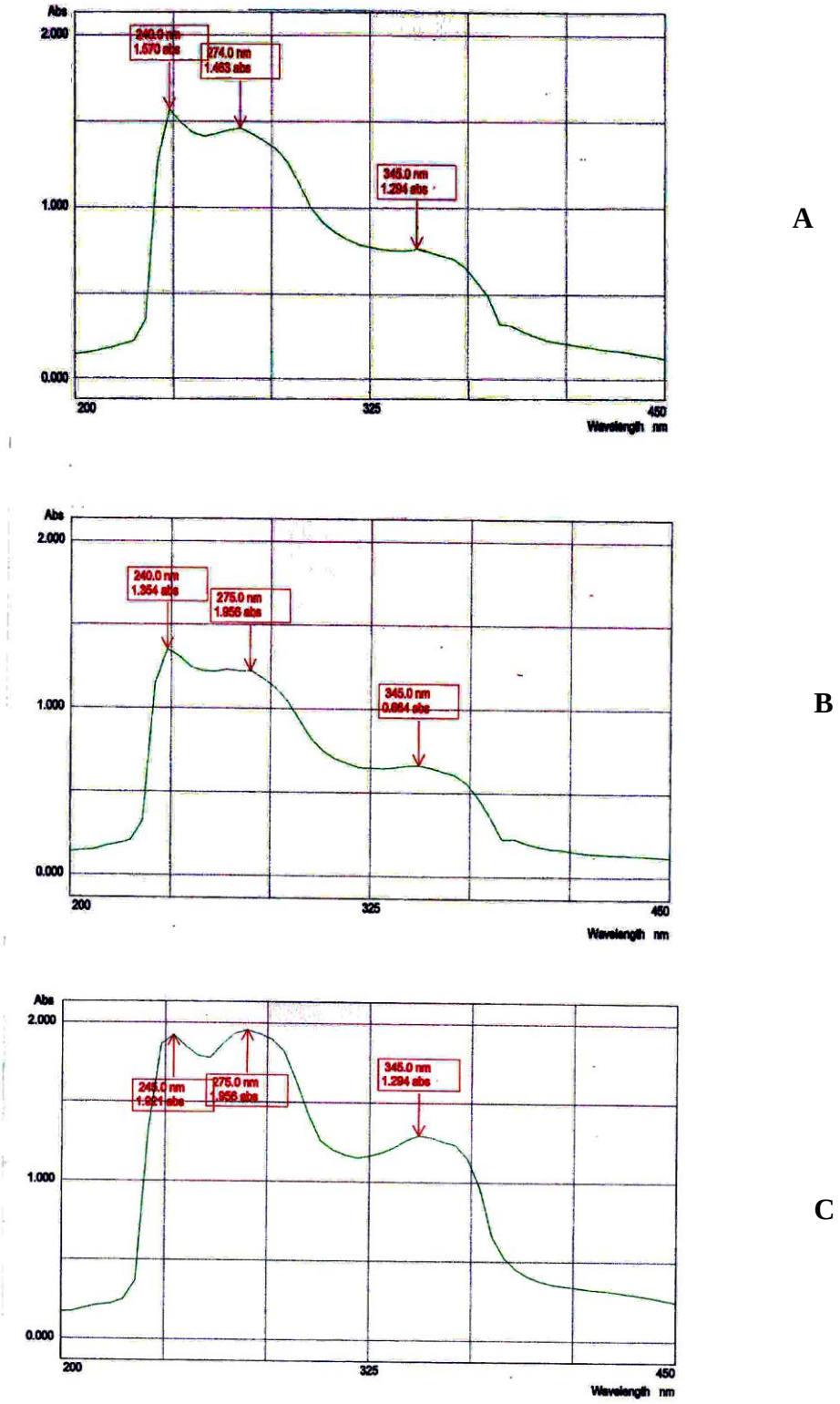


Figure 2. UV spectra for the flavonoids from steams (A), leaves (B) and flowers (C).

The maximum of absorption from the experimental UV spectra corresponds from those given in literature data for flavones or flavonols in aglyconic or heterosidic form [7,8].

But, comparing the experimental bidimensional chromatogram to the standard chromatogram of Marbry and the results of color tests on the elutes, it had already been established that the flavonoid is an aglycon, which is similar in all studied vegetal organs.

As it can be seen in **(Figure 3),** the three IR spectra are quite similar. It was conclu-ded once again that the flavonoidic compound is the same in all the organs of the plant.

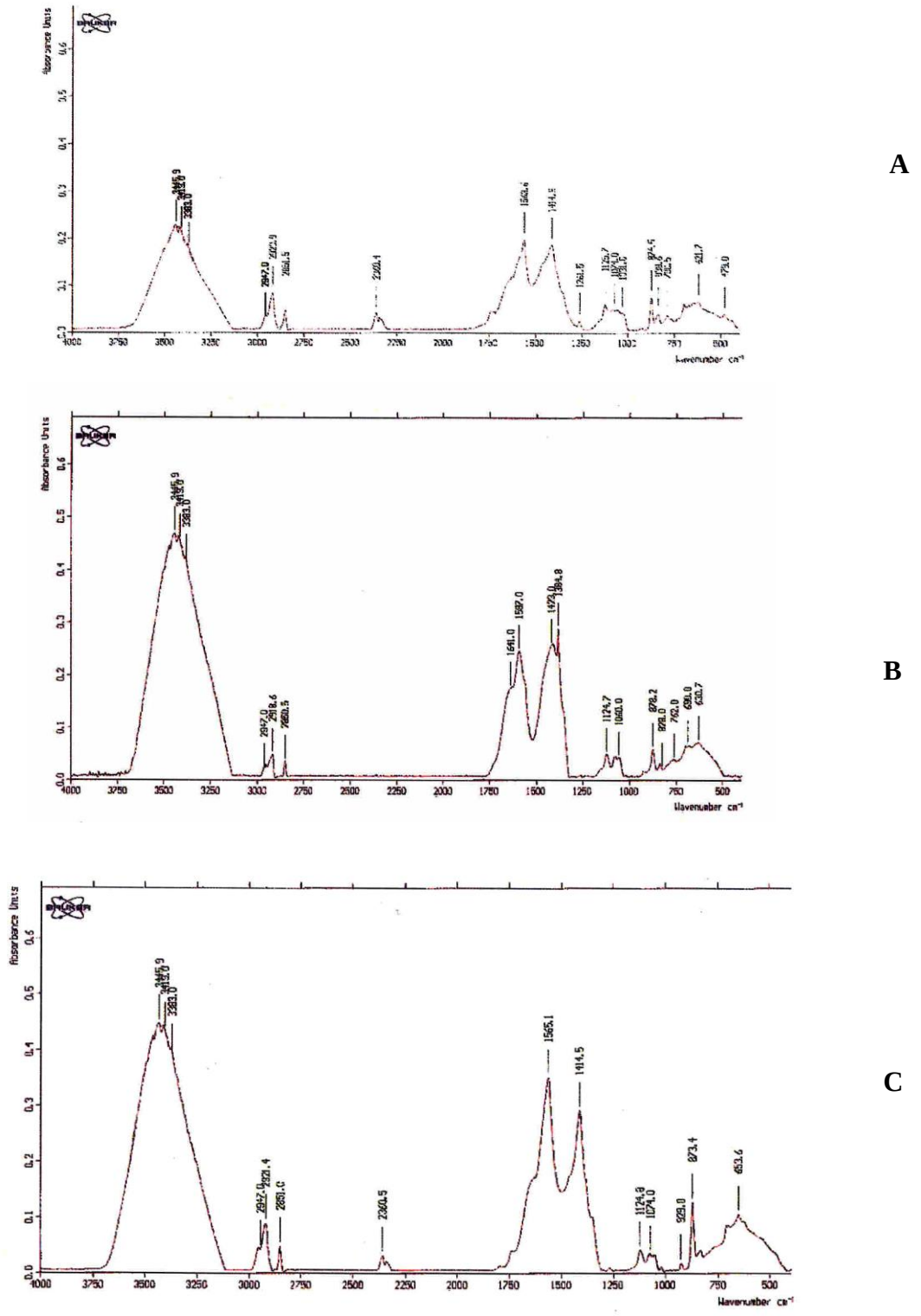
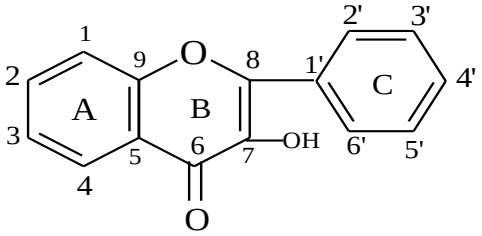


Figure 3. IR spectra for the flavonoids from steams (A), leaves (B) and flowers (C).

The interpretation of the IR spectra (realized from the crystals obtained from the elutes of the bidimensional chromatograms) allowed the following conclusions:

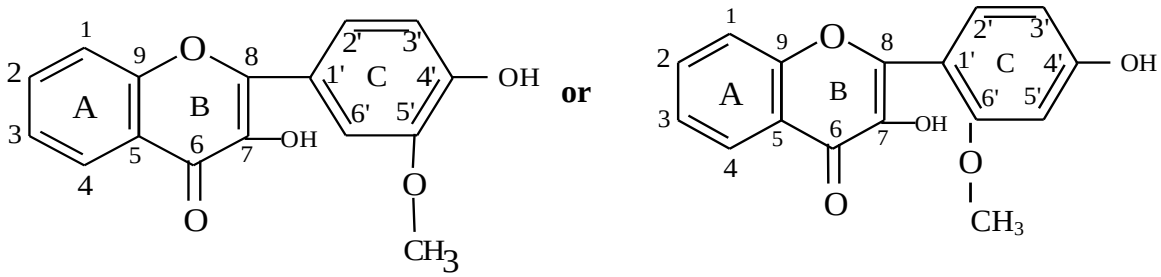
- $\nu = 2850\ cm^{-1}$ indicates the presence of symmetric – O-CH_3 ;
- $\nu = 3383\ cm^{-1}$ and $\nu = 3419\ cm^{-1}$ indicate the presence of associated -OH;
- $\nu = 3445\ cm^{-1}$ indicates the presence of associated –OH;
- $\nu = 1641\ cm^{-1}$ indicates the presence of quinonic group;
- $\delta_{C-H} = 692\ cm^{-1}$ (characteristically for four C-H associated bonds) might correspond to those of $C_2 \div C_4$ from the ring A from the flavonolic structure



- $\delta_{C-H} = 828\ cm^{-1}$ (characteristically for two C-H associate bonds) might correspond to those from C'$_2$ ÷ C'$_3$, C'$_3$-C'$_4$, C'$_4$-C'$_5$ or C'$_5$-C'$_6$ of ring C from the flavonolic structure;
- $\delta_{C-H} = 692\ cm^{-1}$ (characteristically for a single C-H bonds) might correspond to those from $C_2 \div C_4$ of the ring A from the flavonolic structure;

Conclusions

The experimental results presented in this paper allow the final conclusion that the steams, leaves and flowers from *Chelidonium majus L.* contain a single flavonoidic compound, which is a flavonol in an aglyconic form, with a possible chemical structure:



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