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Antioxidant activity of *Chelidonium majus* L. extracts from the Banat county

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Abstract

This paper presents an antioxidant activity evaluation of aqueous and alcoholic *Chelidonium majus L.* extracts by using the DPPH method. The whole raw celandine plant was extracted with water or ethanolic solution (in various concentrations) at room temperature or at reflux and the spectrophotometric time scan in the presence of DPPH solution was done. The best antioxidant activity was obtained for concentrated ethanol hot extracts and diluted ethanol cold extracts, the mean DPPH reaction rate being in the range of 0.5-3 μM/s.

Keywords: *Chelidonium majus* L., celandine, alkaloids, flavonoids, antioxidant activity, DPPH

1. Introduction

The Chelandine (*Chelidonium majus* L.) belong to the Papaveraceae family and it grows in many regions of the world. In Romania it grows on cultivated lands and close to houses. *C. majus* is an herbaceous and perennial plant, with deeply divided leaves and yellow flowers and juice. It grows up to 100 cm [1-3]. The biologically active compounds from celandine can be found especially in roots, but also in leaves and flowers, and belong to the benzophenanthridine and isoquinoline alkaloids [1-15]. The concentration and chemical composition of these alkaloids differ with the vegetation period (up to 4% alkaloids). The main alkaloids from *C. majus* are chelerythrine, sanguinarine (~1%), chelidonine (~0.8%), coptisine, homochelidonine, protopine, allocryptopine, berberine, chelidamine, tetrahydrocoptisine, hydroxychelidonine, methoxychelidonine, oxychelidonine, chelamidine, chelamine, chelidoxanthine, chelilutine, chelirubine, corisamine, dihydrosanguinarine, hydroxysanguinarine, magnoflorine, sparteine, and stylopine. Other compounds identified in *C. majus* are organic acids (1.4-4.3%) like chelidonic, citric, formic, malic, nicotinic, succinic acids, alcohols (chelidoniol), choline, ergosterol, histamine, methylamine, nonacosanol, spinasterol, tyramine, saponosides, flavonoids [2].

Celandine was used from ancient times for treatment of some liver diseases. Systematic research on the *C. majus* pharmacological properties starts in 19th century, but even today the traditionally recipes exceed the number of drug formulations which contain bioactive compounds from *C. majus*. All of these formulations are often used for the treatment of wart and other skin affections, but also for the spleen, choler, and liver diseases [1,2].

Pure alkaloids from *C. majus* have higher toxicity and various pharmacological actions (even synergistic or antagonistic action in the alkaloid mixture) [1,2,6]. Chelidonine and homochelidonine have analgesic and antispasmodic properties

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(like morphin), and breath stimulating action [2,6]. Protopin act on the vasomotor nervous centre and sanguinarin has excitant action on the medullary centres, stimulating the intestinal peristalsis and conduct to increase the gastric, pancreatic, hepatic, and intestinal secretion. Chelerythrine is a local irritant, lowering the arterial pressure and stimulating the peristalsis and uterine contractions [2]. Chelidonine diminish the tonus of smooth muscle and has antispastic properties (like papaverine). *C. majus* extracts have antibiotic and antifungal properties (*e.g.* against *Stafilococcus*, *Streptococcus, Escherichia, Candida, Bacillus subtilis*), as well as antiviral activity [2]. Sanguinarin has also antitumoral effects (cholchicine type), cytostatic properties (celandine is recommended in the treatment of skin cancer) [2,11]. Semisynthetic compounds are used in antitumoral protocols. Aqueous or diluted hydroalcoholic *C. majus* extracts stimulating the bile and liver activity, healing the hepatic cell, and increase the external pancreatic secretion, being used in hepato-biliary afections, cholecystopathy, and in the treatment of initial hepatic cyrosis [2,4,5,9].

In this paper we try to evaluate the antioxidant capacity (the overall antioxidant activity using the DPPH method) of *Chelidonium majus* L. (from the Banat county) extracts obtained by using aqueous and alcoholic solvents with different polarities.

2. Materials and Method

Materials. Celandine (*Chelidonium majus* L.) was harvested from the west side of Romania (Banat County) in April-June 2007 and the whole raw plant (grounded) was used for extraction. Ethanol 96% (v/v) used for extraction was achieved from Chimopar, Bucureşti and DPPH (2,2-diphenyl-1-pycrylhydrazyl) used for antioxidant activity determination was purchased from Merck&Co, Inc. (analytical grade, >99%).

Obtaining the Chelidonium majus L. extracts. Approximately 5 g grounded sample was introduced in a 100 ml extraction flask togeter with 20 ml extraction solvent (dethanol-water solutions at various concentrations, Table 1). A reflux condenser was attached to the extraction flask (equipped with a magnetic stirrer system) and the extraction was achieved at 25ºC (code *Cm_C_1-4*) or at the ethanol temperature reflux (code *Cm_H_5-8*) for one hour or 0.5 hours, respectively. The extraction mass was filtered, washed with 4 ml ethanol-water solution, and the resulted extracts were subjected to the antioxidant activity evaluation.

Antioxidant activity evaluation. Antioxidant activity of the *Chelidonium majus* L. extracts was achieved by using the DPPH method. Spectrophotometric analysis was realized by using a Perkin Elmer Lambda EZ Series spectrophotometer and the acquisition and handling the data a PESSW ver. 1.2, Revision E soft was used. The spectrophotometer cuvette contains 2.4 ml 96% ethanol, 0.3 ml extract (or diluted extract), and 0.3 ml 1mM DPPH ethanolic solution. The recording of the absorbance was realized at 517 nm for 300 s and ethanol 96% (v/v) was used as reference.

Table 1. Extraction conditions for *Chelidonium majus* L. samples

No	Code	m_{sample} (g)	$V_{solvent}$ (ml)	$c_{ethanol}$ (%, v/v)	$V_{extract}$ (ml)
1	Cm_C_1	5.00	20.0	96	23.0
2	Cm_C_2			60	23.4
3	Cm_C_3			20	23.2
4	Cm_C_4			0	24.0
5	Cm_H_5			96	24.0
6	Cm_H_6			60	24.2
7	Cm_H_7			20	24.4
8	Cm_H_8			0	24.5

3. Results and Discussion

It is well known that the flavonoidic compounds and some alkaloids have antioxidant activity due to the presence of the phenolic hydroxyl groups on structures (phenols and polyphenols). *Chelidonium* species contain such compounds and the best method to separate them is ethanolic extraction. In order to evaluate the best extraction conditions for a high antioxidant activity of the *Chelidonium majus* L. samples from Banat county different ethanol concentration and temperature extraction were used (Table 1).

A rapid and better method used for antioxidant activity evaluation is the DPPH method; the spectrophotometric analysis of the DPPH consumption can be achieved and the then the rate of DPPH reaction (which revealed the reaction rate of phenolic alkaloids/flavonoids/other polyphenols) can indicate the antioxidant activity profile of the samples. In order to calculate the instantaneous DPPH concentration a DPPH calibration curve (*Absorbance = -0.01 + 10.87·concentration(mM)*) was obtained (Figure 1).

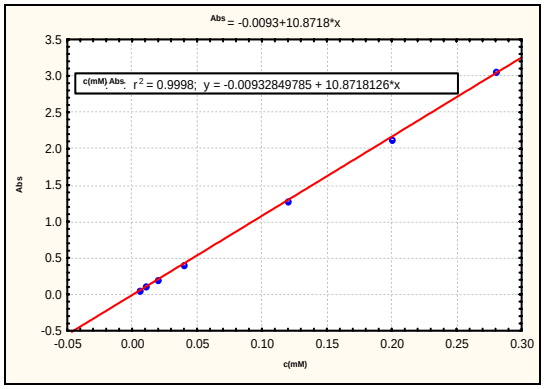

Figure 1. Calibration curve for DPPH (at 517 nm)

Time monitoring of the DPPH concentration in the presence of the *Chelidonium majus* L. extracts (even for nondiluted samples or diluted ones) indicates that the variation was logarithmic in most of the cases (Figures 2, 3).

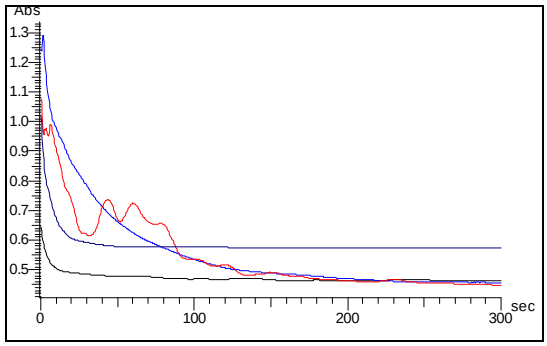

Figure 2. Spectrophotometric time scan in the case of the undiluted (black and dark blue) and two times diluted (red and light blue) of *Cm_C_3* and *Cm_C_4* cold extracts, respectively

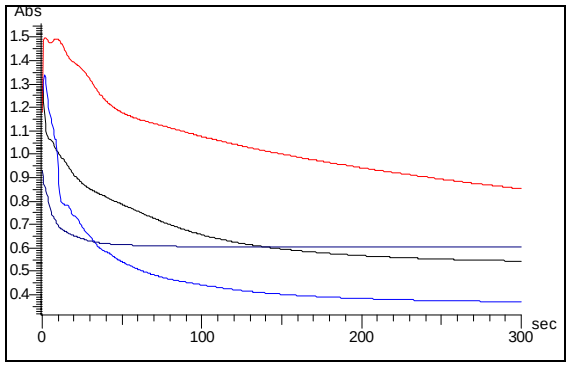

Figure 3. Spectrophotometric time scan in the case of the undiluted (black and dark blue) and two times diluted (red and light blue) of *Cm_H_5* and *Cm_H_6* hot extracts, respectively

In order to evaluate the antioxidant capacity of the extracts, which can be used for sample comparations, the *Relative absorbance (A%) vs. Time (s)* dependences were obtained. The relative absorbance (*A%*) was calculated as the percent ratio of the momentan and initial absorbance of the above mentioned mixture (1:1:8 for sample:DPPH solution:ethanol). Thus, if the *A%* is lower, the antioxidant activity of the sample is higher.

For the cold extract samples the antioxidant activity was lower for all samples obtained with concentrated ethanol (*A%* > 60%), with the exception of non-diluted ethanol 96% extract (*Cm_C_1*), for which the antioxidant activity was lower than 40% (Figure 4). It is possible that this evaluation of the antioxidant activity was difficult due to the high reaction rate of the existing flavonoids/alkaloids.

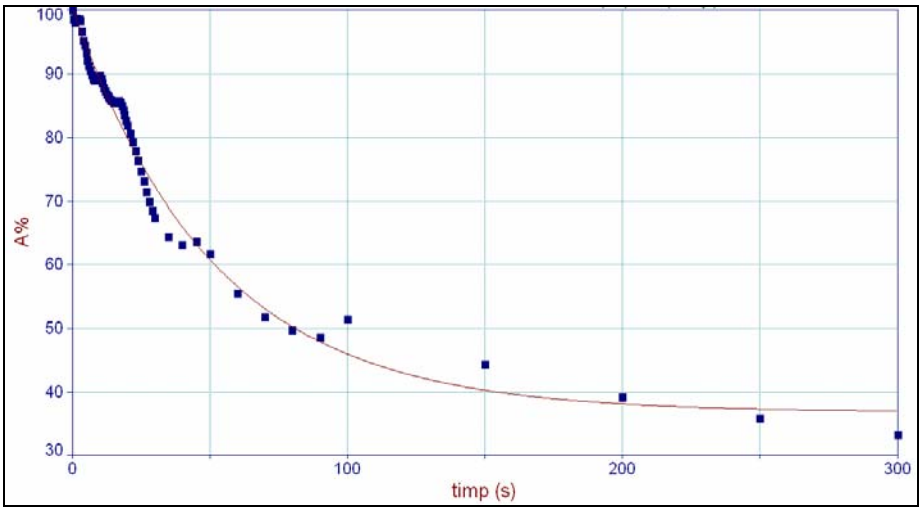

Figure 4. Antioxidand activity (*A%*) of the *Chelidonium majus* L. 96% ethanolic cold extract sample

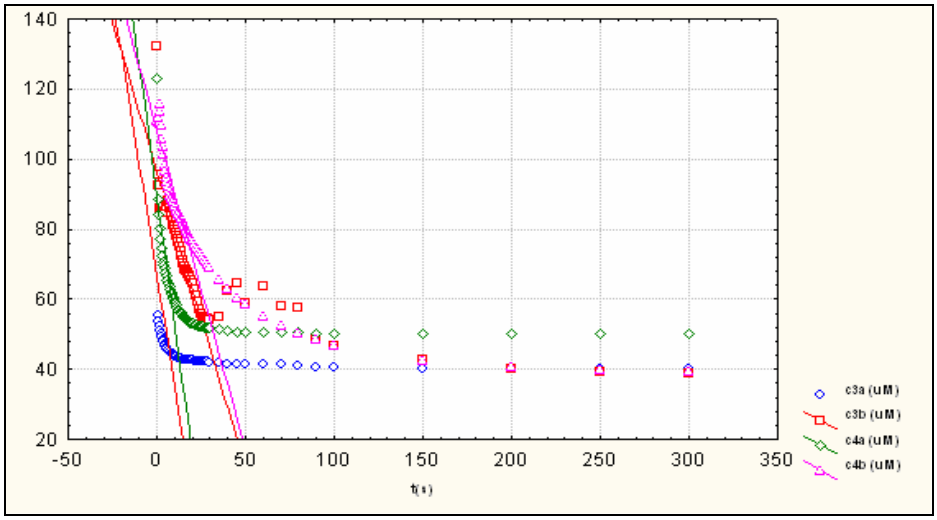

Figure 5. DPPH concentration (μM) *vs.* reaction time (s) in the presence of undiluted and diluted *C. majus* cold extracts (in EtOH 20% and water; code *Cm_C_3* and *Cm_C_4*)

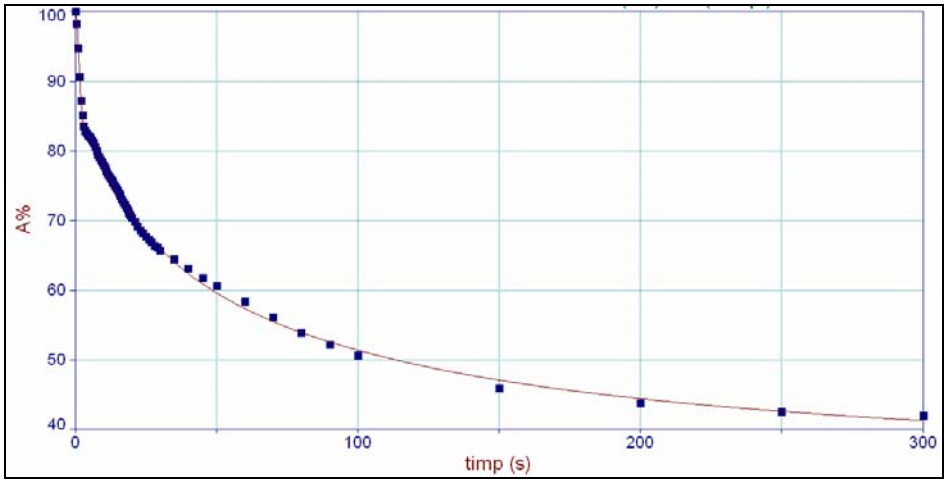

Figure 6. Antioxidand activity (*A%*) of the *Chelidonium majus* L. 96% ethanolic hot extract sample

Tabel 2. Mean reaction rate of DPPH in the case of *Chelidonium majus* L. cold extracts

No	Code	Description	$\bar{v}$ (µM/s)
1	Cm_C_1	*C.majus* extract (EtOH20%), undiluted (a)	3.2
2	Cm_C_2	*C.majus* extract (EtOH20%), diluted (b)	1.7
3	Cm_C_3	*C.majus* extract (water), undiluted (a)	3.7
4	Cm_C_4	*C.majus* extract (water), diluted (b)	1.8

Table 3. Mean reaction rate of DPPH in the case of *Chelidonium majus* L. hot extracts

No	Code	Description	$\bar{v}$ (µM/s)
1	Cm_H_5	*C.majus* hot extract (EtOH96%), undiluted	1.3
2	Cm_H_5a	*C.majus* hot extract (EtOH96%), diluted (a)	0.6
3	Cm_H_6	*C.majus* hot extract (EtOH60%), undiluted	2.1
4	Cm_H_6a	*C.majus* hot extract (EtOH60%), diluted (a)	2.0
5	Cm_H_7	*C.majus* hot extract (EtOH20%), undiluted	1.5
6	Cm_H_7a	*C.majus* hot extract (EtOH20%), diluted (a)	3.0
7	Cm_H_8	*C.majus* hot extract (water), undiluted	2.9
8	Cm_H_8a	*C.majus* hot extract (water), diluted (a)	2.5

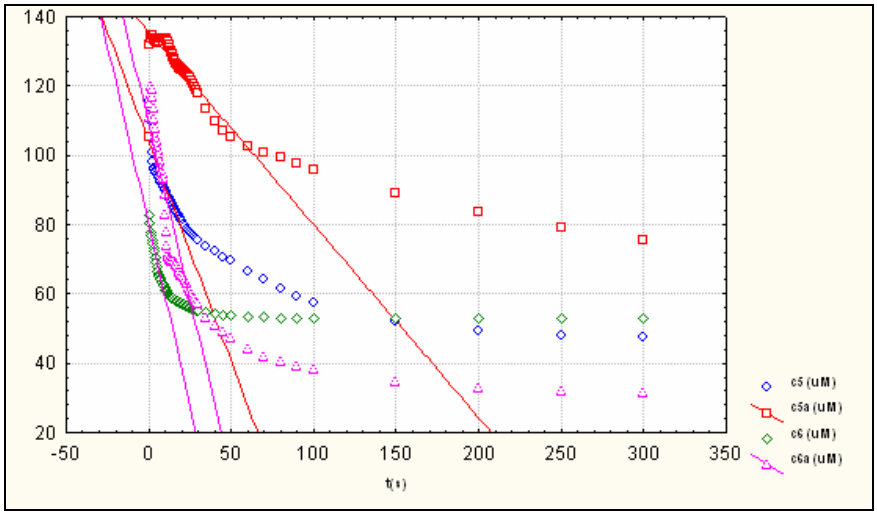

Figure 7. DPPH concentration (µM) *vs.* reaction time (s) in the presence of undiluted and diluted *C. majus* hot extracts (in EtOH 96% and EtOH60%; code *Cm_H_5* and *Cm_H_6*)

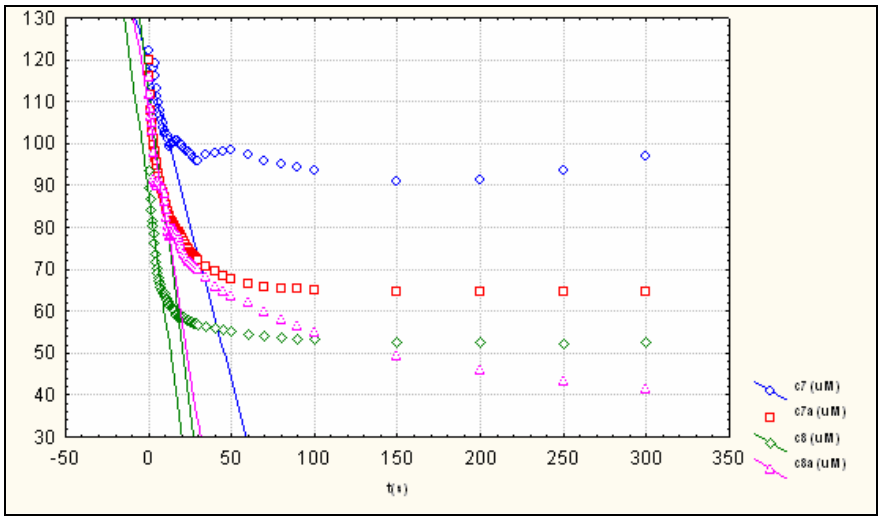

Figure 8. DPPH concentration (µM) *vs.* reaction time (s) in the presence of undiluted and diluted *C. majus* hot extracts (in EtOH 20% and water; code *Cm_H_7* and *Cm_H_8*)

In the case of diluted ethanol cold extracts the antioxidant activity was little bit higher, probable to the presence of a higher content of flavonoside compounds (with water-soluble saccharide moieties): *A%* of 30% for the case of 20% ethanolic extract and 40% for the aqueous extract.

By using the momentan absorbance of the reaction mixture and the calibration curve for DPPH the mean reaction rate of DPPH on the pseudolinear side of the *Concentration (μM) vs. Time (s)* can be evaluated. The mean reaction rate for DPPH is expressed as:

$$\bar{v} = -\frac{\Delta c_{DPPH}}{\Delta t} \quad (\mu M/s)$$

where $\bar{v}$ is the mean reaction rate of DPPH on the pseudolinear side of the curve (μM/s), and $-\Delta c / \Delta t$ is the ratio between the DPPH concentration and time on the corresponding range. Thus, for the 20% ethanol and water extracts the DPPH consumption rate was similar for the undiluted samples (3.2 and 3.7 μM/s, respectively, Figure 5 and Table 2), and approximately a half for the diluted ones (1.7 and 1.8 μM/s, respectively).

For the *C. majus* hot extracts, the antioxidant activity (expressed as percentage of the relative absorbance, *A%*) was in the range of 30% to 65%, the best being observed for the case of ethanol 96% and ethanol 60% extracts (40% and 30%, respectively, Figure 6). For the ethanol 20% and water extracts these values were higher (lower antioxidant activity, 50-60%).

Spectrophotometric time scan analysis of the *C. majus* hot extract samples in the presence of DPPH solution revealed that the DPPH reaction rate was lower even the overall antioxidant activity was higher comparatively with the cold extracts. These DPPH reaction rates were little bit lower (0.6-2 μM/s) in the case of ethanol 96% and 60% extracts comparatively with the ethanol 20% and water extracts (Table 3 and Figures 7, 8). This can be due to the presence of flavonoids (aglycons) and phenolyc alkaloids with slower reactivity, but with a high antioxidant capacity; the more hydrophyllic solutions (ethanol 20% and water) extracts a higher concentration of flavonosides (which contain saccharide moieties), with a higher consumption rate.

4. Conclusion

The study regarding the obtaining, analysis and antioxidant activity evaluation of *Chelidonium majus* L. cold and hot extracts revealed that the DPPH method is a good method for the antioxidant activity evaluation (due to the reproductibility and the impossibility of spectrophotometric interferences between DPPH and phenolic alkaloids/flavonoids absorption zones). Relatively high antioxidant activity was observed in the case of cold extracts obtained with more hydrophyllic solvents (ethnaol 20% and water) and in the case of hot extracts obtained with concentrated ethanol (60% and 96%). The mean DPPH reaction rate (as well as the phenolic alkaloids/flavonoids overall reaction rate) was in the range of 0.5-3 μM/s; higher reaction rate was calculated for the diluted ethanol and water cold extracts, which probable contain a high concentration of flavonosides, and a lower reaction rate was observed in the case of concentrated ethanol hot extracts, which contain a higher concentration of flavonoids (aglycones) and phenolic alkaloids, more soluble in these solvents.

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