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EXPERIMENTAL PAPER

# Comparison of antioxidant activities of fractionated extracts from seedlings and herb of *Chelidonium majus* L. using DPPH, ABTS and FRAP methods

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S u m m a r y

**Introduction:** Our study is a part of a trend of studies on the antioxidative properties of *Chelidonium majus* extracts or their fractions suggesting that antioxidant activities may depend on total flavonoid and/or alkaloid contents. **Objective:** This study focused on the examination of antioxidative activities of full water extract, non-protein fraction and protein fraction of the extract from aerial parts of mature plants and young seedlings. **Methods:** Total flavonoid and alkaloid contents were evaluated by spectrometric methods. Quantitative determination of chelidonine, coptisine, sanquinarine, berberine was made by HPLC-UV. The antioxidative activities were evaluated using **(1)** 2,2-diphenyl-1-picrylhydrazyl (DPPH), **(2)** 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging and **(3)** ferric reducing antioxidant power (FRAP) methods. **Results:** All concentrations of herb extracts exhibited higher antioxidant capacities than extract from seedlings. Two antioxidant tests (DPPH, FRAP) showed that full water extract from herb had the highest antioxidant activity, while its non-protein fraction and protein fraction showed lower antioxidant activity. It was found that the full water extract from herb contained the highest concentrations of flavonoids and alkaloids when compared with other samples. **Conclusion:** Our findings suggest that chelidonine and coptisine especially could be responsible for the observed changes in the extract antioxidant activity, because these alkaloids were determined in the highest concentration in full water extract from herb. It cannot be also excluded that the observed variables values between extracts and their fractions from herb or from seedlings may also be the result of interactions between flavonoids and other chemical compounds.

**Key words**: *Chelidonium majus, greater celandine, herbal extract, antioxidant activity, DPPH, ABTS, FRAP, phytochemical analysis, alkaloids, flavonoids, statistical analysis*

**Abbreviations:**
F1H – full water extract from herb
F2H – protein fraction of extract from herb

F3H – non-protein fraction of extract from herb
F1S – full water extract from seedlings
F2S – protein fraction of extract from seedlings
F3S – non-protein fractions of extract from seedlings

## INTRODUCTION

*Chelidonium majus* L. (CM, greater celandine) belongs to *Papaveraceae* and it is widespread throughout the world, including Europe, Asia, Northwest Africa and North America [1]. The aerial part of this plant contains isoquinoline alkaloids, such as chelidonine, chelerythrine, sanguinarine, berberine, coptisine, and stylopine (more than 30 alkaloids) [2-5]. Moreover, the presence of flavonoids, saponins, organic acids, vitamins, carotenoids, tyramine and several hydroxycinnamic acid derivatives including caffeoylmalic, chelidonic, malic and citric acids has been also reported [1]. Aerial parts of CM (*Chelidonii herba*) are used for the symptomatic treatment of mild to moderate spasms of the upper gastrointestinal tract, minor gallbladder disorders, and dyspeptic complaints such as bloating and flatulence [1]. Moreover, CM is traditionally used in the treatment of skin diseases such as eczema, ringworm, oral infection, pains, and nervous disorders [1, 2, 6]. It has been shown that CM showed an anti-inflammatory, antimicrobial, antiviral, antitumor, analgesic, and antihepatotoxic activities [1, 7]. Recently, it was shown that extracts from CM produced centrally mediated (morphine-like) analgesic action and exerted the anti-inflammatory potential [5].

Oxidative stress can lead to chronic inflammation, which in turn could mediate other chronic diseases including cancer, diabetes, cardiovascular and pulmonary diseases [8], as well as neuropathic pain [9]. Moreover, oxidative stress has been implicated in the pathophysiology of numerous neuropsychiatric disorders such as unipolar affective disorder, schizophrenia [10], and neurodegenerative processes leading to Alzheimer's and Parkinson's diseases [11].

It is known that during chronic inflammation, free radicals are formed in excess and cannot be effectively eliminated from the organism [12, 13]. Moreover, not only cytokines but also reactive oxygen species have been implicated in the cascade of events resulting in inflammatory pain [14] which gives an antioxidant activity a fundamental role in cellular protection during an inflammation process [15].

From naturally occurring compounds the phenolics and flavonoids have been reported to be the most important chemical compounds responsible for the antioxidant capacity [16]. Furthermore, several studies showed that alkaloids also exerted antioxidant properties [17-19]. Up to date there is a little information about antioxidant activities of extracts from CM. In a previous study, Duda-Chodak *et al.* [20] showed that water extract of CM (*infusum*) presented a low antioxidant activity in comparison to other 14 plant extracts. Therefore, evaluation of antioxidant

status of extracts may be a complement to explain the mechanism of anti-inflammatory activity of extracts from *Ch. majus*.

## Aims

The objectives of this study were as follows: (1) to evaluate and compare the antioxidant capacity by three common methods, presented as DPPH, ABTS and FRAP, of extracts of *Ch. majus* such as full water extract, non-protein fraction and protein fraction from of aerial parts of mature plants (herb) and young seedlings, (2) quantitative estimation of chosen alkaloids by HPLC and total flavonoid (expressed as quercetine) and total alkaloid (expressed as chelidonine) contents by spectrophotometric methods. All chemical compounds were analyzed in dry extracts from *Ch. majus*.

## MATERIAL AND METHODS

### Plant material

Plant material of *Chelidonium majus* 'Cynober', were collected from a controlled cultivation at the Institute of Natural Fibres and Medicinal Plants in Poznan, Poland. The field cultivation was established in spring by sowing seeds directly into ground. The herb was harvested during the beginning of flowering.

The cultivation of seedlings in pots was carried out using seeds obtained from the ground cultivation (*Ch. majus* 'Cynober'). Seedlings were collected after 14 days. The raw material was dried at 55–60°C.

### Phytochemical study

#### Preparation of full water extract (F1H), protein (F2H) and non-protein (F3H) fractions of full water extract from herb

A sample of plant material (10 kg of dry herb) was put in cotton bag, which was in turn, fed into the extractor vessel along with demineralized water (45 l). Solvent was pumped from the bottom of the vessel (solvent volumetric flow rate was equal 25 l/min) and was distributed by spray nozzles over the surface of the cotton bag. The material was left in contact with the solvent until equilibrium of the active principle was achieved (1.5 h). Temperature was kept constant at 90°C. After the desired time, the solvent extract was taken out from the bottom discharge valve of the extractor. Pure solvent (45 l) was added into the vessel and the extract was drained out after acquiring equilibrium (1.5 h). Overall, the plant

material was washed two times until it got exhausted. All washes from the extractor were pooled and fed into a evaporator unit where it was concentrated under vacuum to 1/3 volume. This extract from herb was marked as F1H (full water extract) and has been divided into two parts. One part of the F1H was further cooled down to 4°C in a double-jacketed vessel with chilled water circulating between inner and outer walls.

Next, the FT-29 Armfield solid-liquid pilot-plant extraction equipment (UK) was used for extraction of proteins from herb. It consist of an extractor vessel with perforated bottom (stainless steel sieve) for holding the material to be extracted, a gear pump for pumping the solvent, vacuum evaporator unit and a storage vessel.

The proteins were precipitated from the second part of the F1H by adding four volumes of ice-cold acetone (-20°C, 30 min). The supernatant (non-protein fraction) was decanted and the precipitate was rinsed with cold acetone and centrifuged (4500 g, 4°C, 20 min). Acetone was discarded, and pellet was suspended in distilled water. Before freeze drying process, the protein fraction was put to compaction using a rotary vacuum evaporator (Laborota, Heidolph, Germany) to remove residual acetone. The fraction was frozen at -50°C and freeze-dried in a Freeze Dryer Beta 1–16 (Christ, Germany) over a period of 36 h. Protein fraction from extract of herb was marked as F2H. To produce non-protein fraction from herb, the supernatant containing acetone was concentrated on the rotary vacuum evaporator and freeze-dried as above. Non-protein fraction from extract of herb was marked as F3H. In summary, the full water extract (F1H), protein fraction (F2H) and non-protein fraction (F3H) were lyophilized at -50°C and those dry samples (extracts) were used to determine the bioactive compounds.

**Preparation of full water extract (F1S), protein (F2S) and non-protein (F3S) fractions of full water extract from seedlings**

Small-scale solid-liquid extraction system equipped with double wall, stainless steel vessel (4 l), stainless steel sieve, vapor condenser and gear pump was loaded with ca. 200 g of dry seedlings. Extraction was conducted under the same conditions as described above (water as a solvent, and solvent/raw material ratio of 10:1). The full water extract, protein as well as non-protein fractions of extract from seedlings were marked as F1S, F2S and F3S, respectively.

**Determination of total flavonoid content expressed as quercetine**

Total flavonoids were analyzed according with European Pharmacopoeia 6 (monography of birch leaf) [21]. Sample: 0.5–1.0 g of dry full water extract or its fraction was weighted, extracted with acetone under reflux condenser. Next, acetone fraction was extracted with ethyl acetate. Combined ethyl acetate fractions were reacted with aluminium chloride reagent and an absorbance was measured. The absorbance was measured by comparison with the compensation liquid at $\lambda$=425 nm.

### Determination of total alkaloid content expressed as chelidonine

Total alkaloids were analyzed according with European Pharmacopea 6 (monography of greater celandine) [21]. Sample preparation was as follows: 0.75 g of dry full water extract or its fraction was weighted, extracted with 12% acetic acid. The sample was shaken with ammonium and methylene chloride. An organic fraction was evaporated to dryness. The residue was dissolved in 96% ethanol, next it was transferred quantitatively and dissolved with sulphuric acid. The sample was reacted with chromotropic acid sodium salt solution in sulphuric acid (10 g/l). The absorbance was measured at $\lambda=570$ nm by comparison with the compensation liquid.

### Determination of alkaloids by HPLC

An identification method of alkaloids by liquid chromatography was based on isolation alkaloids method from European Pharmacopoeia 6 (monography of greater celandine) [21]. Sample: 0.5–1.0 g of dry full water extract or its fraction was weighted, extracted with 12% acetic acid. The sample was shaken with ammonium and methylene chloride. An organic fraction was evaporated to dryness. The residue was dissolved in methanol and analyzed by liquid chromatography. HPLC-DAD analysis was performed on a Agilent 1100. Separation of methanolic sample was prepared on ZORBAX Poroshell 120 SB-C18 (Agilent) $3\times100$ nm (2.7 $\mu$m). Column temperature was 40°C. The volume of injection was 50 $\mu$l. A gradient mixture of phase A: 30 mM ammonium formate (pH=2.8) and phase B: acetonitrile: methanol 14.7:18.0 (*v:v*) were used as eluent, starting from 20% phase B to 60% phase B in 16 min. The flow-rate was 0.50 ml/min. Peaks were identified by the addition of standard solutions and by UV-VIS spectra.

Quantitative determination for coptisine and chelidonine were performed at $\lambda=240$ nm, for sanguinarine and chelerythrine - $\lambda=280$ nm and for berberine – $\lambda=345$ nm by an external standard method. Standard solutions were prepared in methanol [5].

## ANTIOXIDANT ASSAYS

### Scavenging ability measured by the DPPH radical assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the extract was measured according to the modified method of Brand-Williams *et al.* [22]. 0.5 g of dry full water extracts or their fractions were dissolved in 40% ethanol v/v (50 ml) (basal solutions). Next, 0.5, 1.0, 2.0, 2.5 ml of basal solutions were placed in volumetric flasks (10 ml) and dissolved in 40% ethanol *v/v* to 10 ml, next 0.1 ml of previously prepared dilutions of the extracts was mixed with 3.9 ml of $6\times10^{-5}$ M/l ethanolic solution of the DPPH radical. After 30 min of incubation at room temperature (in a dark place), a decrease in the absorbance at $\lambda=515$ nm

was measured using a spectrophotometer (UV-VIS, Cintra 20, GBC Scientific Equipment Pty Ltd, Australia). The scavenging capacity of the extracts was compared with that of trolox (y=0.50981+219.29 x, r=0.999) and expressed as the trolox equivalent antioxidant capacity (TEAC). All determinations were performed in triplicate.

**Scavenging ability measured by stable ABTS radical cation**

Free radical scavenging activity of the plant extract was determined according to the modified method of Re *et al.* [23]. The $ABTS^+$ solution (5.5 ml) was diluted with ethanol (42 ml) to an absorbance at λ=734 nm ($A_0$). 0.5 g of dry full water extracts or their fractions were dissolved in 40% ethanol *v/v* (50 ml) (basal solutions). Next, 0.25, 1.0, 1.25, 1.5, 1.75 ml of basal solutions were placed in volumetric flasks (10 ml) and dissolved in 40% ethanol *v/v* to 10 ml, next 30 μl of previously prepared dilutions of the extracts was mixed with 3.0 ml of the diluted $ABTS^+$ solution. After exactly 6 min (*At*) absorbance was measured at λ=734 nm.

The scavenging capacity of the extracts was compared with that of trolox (y = -0.3021+6.2506 x, r=0.996) and expressed as the trolox equivalent antioxidant capacity (TEAC). All determinations were performed in triplicate.

**Ferric reducing/antioxidant power assay (FRAP)**

The total antioxidant potential of the extract was determined according to Benzie and Strain [24] with some modification, using the ferric reducing ability of plasma FRAP assay. 0.5 g of dry full water extracts or their fractions were dissolved in 40% ethanol *v/v* (50 ml) (basal solutions). Next, 0.125, 0.25, 0.50, 0.75, 1.00 ml of basal solutions were placed in volumetric flasks (10 ml) and dissolved in 40% ethanol *v/v* to 10 ml, next 3.00 ml of previously prepared dilutions of the extracts was mixed with 3.0 ml of freshly prepared FRAP reagent and 0.3 ml of water, and it was allowed to react for 4 min in a water bath (37°C). Using this method, we measured the changes in the absorbance at λ=593 nm after decolorization of blue colored product ($Fe^{2+}$ – tripyridyltriazine complex) as a result of the activity of extracts. The results were corrected for dilution and expressed in μM $Fe^{2+}$/l, and compared with that of trolox (y= -19.41+2.0973 x) and expressed as the trolox equivalent antioxidant capacity (TEAC). FRAP values were calculated on the basis of the calibration curve for $FeSO_4$:

$$\text{FRAP } [\mu\text{M/l}] = \text{c x } 34$$

where: c [μM/l] = 494.67 x A – 5,30
34 – dilution
All determinations were performed in triplicate.

## Statistical analysis

All values were expressed as means ± SEM. The statistical comparison of results was carried out using one-way analysis of variance (ANOVA) followed by Tuckey's post-hoc test for detailed data analysis. The level of statistical significance was set at $p<0.05$.

*Ethical approval: The conducted research is not related to either human or animal use.*

# RESULTS AND DISCUSSION

## Phytochemical analysis

The highest concentrations of these group of chemical compounds (total flavonoid content calculated as quercetin) were determined as follows: F1H > F2H > F3H > F1S > F2S (tab. 1). One-way ANOVA ($F(4,10) = 67.9$, $p<0.0001$) and post hoc analysis revealed significant differences in F1H *vs.* F2H ($p<0.05$), F1H *vs*. F3H ($p<0.05$), F1H *vs.* F1S ($p<0.05$), F2H *vs.* F2S ($p<0.05$).

**Table 1.**

Summary of total flavonoid content, total alkaloid content and detailed determination of chosen alkaloids in extracts by HPLC-UV

| Extract | Total flavonoids expressed as quercetin [g/100 g] | Total alkaloids expressed as chelidonine [g/100 g] | Chelidonine [mg/100 g] | Coptisine [mg/100 g] | Sanquinarine [mg/100 g] | Berberine [mg/100 g] | Chelerythrine [mg/100 g] |
|---|---|---|---|---|---|---|---|
| F1H | 0.32 ± 0.06 | 1.74 ± 0.38 | 110 ± 7 | 629 ±2 | n.d. | 23.4 ± 2.0 | 0.67 ± 0.1 |
| F2H | 0.19 ± 0.02 | 1.13 ± 0.35 | 59 ± 2 | 509 ± 1 | n.d. | 17.1 ± 1.0 | 1.16 ± 0.1 |
| F3H | 0.18 ± 0.01 | 1.46 ± 0.39 | 134 ± 4 | 404 ± 2 | 19.02 ± 1 | 17.7 ± 1.0 | 0.29 ± 0.1 |
| F1S | 0.004 ± 0.001 | 0.48 ± 0.03 | 55 ± 1 | 100 ± 4 | 18.99 ± 1 | 37.1 ± 3.0 | 5.15 ± 0.1 |
| F2S | 0.001 ± 0.001 | 0.08 ± 0.02 | 16 ± 1 | 30 ± 1 | 19.05 ± 1 | 7.4 ± 1.0 | 1.16 ± 0.1 |
| F3S | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |

n.d. – not determined,
Data are expressed as the means ± SD (three replicates)
F1H – full water extract from herb
F2H – protein fraction of extract from herb
F3H – non-protein fraction of extract from herb
F1S – full water extract from seedlings
F2S – protein fraction of extract from seedlings
F3S – non-protein fractions of extract from seedlings

Stancic-Rotaru *et al.* [25] showed that the concentration of flavonoids was the highest in the initial stage of flowering and it was shown concentration of

flavonoids in the range of 7.12 to 291.58 mg/g (calculated as rutin) in all phenological phases [26]. Kaempherol, quercetol, rutoside were identified in 70% ethanolic extract from mature aerial parts of *Ch. majus* [27].

Moreover in our study, the extracts contained different concentration alkaloids (F3H > F1H > F2H > F1S > F2S) (tab. 1). Statistical analysis of total alkaloid content (ANOVA $F(4,10)= 16.9$, $p<0.0001$) showed revealed significant differences in F1H *vs.* F1S ($p<0.05$), F1S *vs.* F2S ($p<0.05$), F2H *vs.* F2S ($p<0.05$) and no statistical differences in F1H *vs.* F2H ($p>0.05$), F1H *vs.* F3H ($p>0.05$), F2H *vs.* F3H ($p>0.05$). Statistical analysis of HPLC results for chelidonine (ANOVA $F(4,10)= 467.0$, $p<0.0001$), coptisine (ANOVA $F(4,10)= 38902$, $p<0.0001$) and berberine (ANOVA $F(4,10)= 111.3$, $p<0.0001$) revealed significant differences between the samples: F1H *vs.* F2H ($p<0.05$), F1H *vs.* F3H ($p<0.05$), F2H *vs.* F3H ($p<0.05$), F1H *vs.* F1S ($p<0.05$), F1S *vs.* F2S ($p<0.05$), F2H *vs.* F2S ($p<0.05$). The highest concentrations of chelidonine were shown as follows: F3H > F1H > F2H > F1S > F2S (tab. 1) and F1H > F2H > F3H > F1S > F2S for coptisine and F1S > F1H > F3H > F2S > F2H for berberine (tab. 1). In a case of statistical analysis of HPLC results for sanguinarine (ANOVA $F(4,10) = 0.01$, $p>0.990$) showed no statistical differences in F1H *vs.* F1S ($p>0.05$). Moreover, statistical analysis (ANOVA $F(4,10) = 1165.0$, $p<0.0001$) for chelerythrine showed significant differences between all samples with exception in F2H *vs.* F2S ($p>0.05$) (tab. 1).

Previous HPLC analysis of isoquinoline alkaloids in alcoholic extracts from aerial parts of mature *Ch. majus* [28] showed lower levels of alkaloids in comparison with our results (tab. 1). Kursinszki *et al.* [28] calculated alkaloid content in extracts from the plant using solvents containing different concentrations of ethanol (40, 70, 90% *v/v*). The highest concentrations of alkaloids (90% ethanol) were as follows: chelidonine (28 mg/100 g), coptisine (354 mg/100 g), sanguinarine (22 mg/100 g), berberine (11 mg/100 g). In our full water extract (F1H), level of chelidonine was 3.92 times higher, similarly as coptisine (1.77 times) and berberine (2.11 times). In our study, sanguinarine and protopine were not detected.

## Antioxidant activity

The antioxidant activities of extracts were tested for: DPPH radical scavenging (2,2-diphenyl-1-picrylhydrazyl); inhibition of free radical ABTS (2,2'-azino-bis 3 ethylbenzo-thiazoline-6-sulfonic acid); and capacity to reduce metal iron (ferric reducing antioxidant power, FRAP). ANOVA analysis revealed significant differences in the antioxidant activity of various concentrations of the CM extracts using the DPPH radical model ($F(4,10)=1657.1$; $p=0.000$ for F1S; $F(4,10)=95.6$, $p=0.000$ for F2S, $F(4,10)=789.8$, $p=0.000$ for F3S and $F(4,10)=10575$, $p=0.000$ for F1H, $F(4,10)=7260.9$, $p=0.000$ for F2H, $F(4,10)=4341.6$, $p=0.000$ for F3H) (tab. 2). The DPPH radical scavenging capacity of the extracts was found to increase in a dose-dependent manner. All extracts from herb of CM showed higher activity in

reduce the stable radical DPPH in comparison to extracts from seedlings for all tested concentrations (tab. 2). Detailed Tukey's post-hoc analysis showed that the F1H demonstrated a higher antioxidant activity and this effect was significantly different to that of the F2H (ranging from 0.1 to 0.25 mg/0.1 ml, $p<0.05$) and the F3H (in range 0.05 to 0.2 mg/0.1 ml, $p<0.05$). Effect observed for F1H corresponded with higher content of total flavonoids (expressed as quercetin) (tab. 1). Analysis of activity of the extracts from seedling showed that F3S demonstrated a higher antioxidant activity in comparison with F1S and F2S, according to results presented in table 2. The trolox equivalent antioxidant capacity (TEAC) values varied from 8.47 to 54.06 nM/l (mean 29.62) for F1S, 4.40 to 33.43 nM/l (mean 20.75) for F2S, 12.51 to 71.73 nM/l (mean 42.68) for F3S, and 61.69 to 301.50 nM/l (mean 196.33) for F1H, 60.42 to 267.87 nM/l (mean 153.59) for F2H, 53.65 to 299.75 nM/l (mean 181.18) for F3H. According to calculation it was shown higher TEAC values in order F1H > F3H > F2H > F3S > F1S > F2S.

**Table 2.**

DPPH radical scavenging capacities of the extracts from *Chelidonium majus* expressed as trolox equivalent

| Extract | Inhibition of DPPH [nM trolox] | | | | | ANOVA |
|---|---|---|---|---|---|---|
| | 0.05 [mg/0.1 ml] | 0.1 [mg/0.1 ml] | 0.15 [mg/0.1 ml] | 0.2 [mg/0.1 ml] | 0.25 [mg/0.1 ml] | |
| F1H | 61.69 ± 0.83 | 140.16 ± 0.89 | 202.56 ± 0.93 | 275.73 ± 0.62 | 301.50 ± 1.34 | $F(4,10) = 10575.0$, $p = 0.000$ |
| F2H | 60.42 ± 0.98 | 104.30 ± 1.16$^{+}$ | 148.06 ± 0.71$^{+}$ | 187.30 ± 1.09$^{+}$ | 267.87 ± 0.59$^{+}$ | $F(4,10) = 7260.94$, $p = 0.000$ |
| F3H | 53.65 ± 0.71$^{+}$ | 130.50 ± 2.66$^{+}$ | 175.86 ± 1.10$^{+}$ | 247.19 ± 0.33$^{+}$ | 299.75 ± 1.34 | $F(4,10) = 4341.64$, $p = 0.000$ |
| F1S | 8.47 ± 0.39 | 14.50 ± 0.60 | 25.37 ±0.28 | 45.69 ± 0.68 | 54.06 ± 0.32 | $F(4,10) = 1657.14$, $p = 0.000$ |
| F2S | 4.40 ± 0.75 | 14.20 ± 0.31 | 22.89 ± 1.15 | 28.83 ± 1.40* | 33.42 ± 1.76* | $F(4,10) = 95.59$, $p = 0.000$ |
| F3S | 12.51 ± 0.42 | 27.65 ± 1.48* | 43.50 ± 0.56* | 58.01 ± 0.8* | 71.73 ± 0.36 | $F(4,10) = 789.84$, $p = 0.000$ |

Data are expressed as the means ± SEM (three replicates)
* statistically significant difference *vs.* F1S (control for F2S and F3S); $p<0.05$
+ statistically significant difference *vs.* F1H (control for F2H and F3H); $p<0.05$
F1H – full water extract from herb
F2H – protein fraction of extract from herb
F3H – non-protein fraction of extract from herb
F1S – full water extract from seedlings
F2S – protein fraction of extract from seedlings
F3S – non-protein fractions of extract from seedlings

ABTS radical scavenging method is one of the most extensively used antioxidant assays in plant extracts. It is applicable to both hydrophilic and lipophilic compounds in plant extracts [29]. Statistical analysis (ANOVA) revealed significant differences in the antioxidant activity of various concentrations of the CM extracts using the ABTS radical model ($F(4,10)=265.0$; $p=0.000$ for F1S; $F(4,10)=211.1$, $p=0.000$ for F2S, $F(4,10)=1054.8$, $p=0.000$ for F3S and $F(4,10)=1564.9$,

$p=0.000$ for F1H, $F(4,10)=1810.3$, $p=0.000$ for F2H, $F(4,10)=3112.3$, $p=0.000$ for F3H) (tab. 3). The ABTS radical scavenging capacity of the extracts was found to increase in a dose-dependent manner. It was shown that all extracts from herb of CM showed higher activity in scavenging the ABTS radical (F3H > F2H) in comparison with extracts from seedlings (F3S > F2S) for all tested concentrations (tab. 3). Detailed Tukey's post-hoc analysis showed that the F3H demonstrated a higher antioxidant activity and this effect was significant different to that of the F1H (ranging from 0.03 to 0.052 mg/0.1 ml, $p<0.05$). Moreover, the extract from seedlings (F3S) showed a higher activity than that of F2S (tab. 3). The trolox equivalent antioxidant capacity (TEAC) values varied from 0.98 to 2.41 $\mu$M/l (mean 1.754) for F1S, 0.72 to 1.80 $\mu$M/l (mean 1.4) for F2S, 0.92 to 2.9 $\mu$m/l (mean 2.16) for F3S, and 1.49 to 4.83 $\mu$M/l (mean 3.77) for F1H, 1.60 to 5.69 $\mu$M/l (mean 3.93) for F2H, 1.41 to 5.90 $\mu$M/l (mean 4.17) for F3H. According to calculation it was shown higher TEAC values in order F3H > F2H > F1H > F3S > F1S > F2S.

**Table 3.**

ABTS radical scavenging capacities of the extracts from *Chelidonium majus* expressed as trolox equivalent

| Extract | Inhibition of ABTS [$\mu$M trolox] | | | | | ANOVA |
|---|---|---|---|---|---|---|
| | 0.0075 [mg/0.03 ml] | 0.03 [mg/0.03 ml] | 0.038 [mg/0.03 ml] | 0.045 [mg/0.03 ml] | 0.052 [mg/0.03 ml] | |
| F1H | 1.49 ± 0.02 | 3.27 ± 0.02 | 4.68 ± 0.01 | 4.60 ± 0.04 | 4.83 ± 0.06 | $F(4, 10) = 1564.947$, $p=0.000$ |
| F2H | 1.60 ± 0.04$^{+}$ | 3.40 ± 0.02$^{+}$ | 4.12 ± 0.06$^{+}$ | 4.84 ± 0.02$^{+}$ | 5.69 ± 0.03$^{+}$ | $F(4, 10) = 1810.320$, $p=0.000$ |
| F3H | 1.41 ± 0.04 | 3.62 ± 0.02$^{+}$ | 4.18 ± 0.01$^{+}$ | 5.74 ± 0.02 | 5.90 ± 0.05$^{+}$ | $F(4, 10) = 3112.274$, $p=0.000$ |
| F1S | 0.98 ± 0.03 | 1.72 ± 0.04 | 1.69 ±0.00 | 1.97 ± 0.00 | 2.41 ± 0.05 | $F(4, 10) = 265.011$, $p=0.000$ |
| F2S | 0.72 ± 0.03 | 1.33 ± 0.02$^{*}$ | 1.37 ± 0.02$^{*}$ | 1.79 ± 0.04 | 1.80 ± 0.03$^{*}$ | $F(4, 10) = 211.114$, $p=0.000$ |
| F3S | 0.92 ± 0.02 | 2.00 ± 0.00$^{*}$ | 2.27 ± 0.01$^{*}$ | 2.71 ± 0.03$^{*}$ | 2.90 ± 0.03$^{*}$ | $F(4, 10) = 1054.824$, $p=0.000$ |

Data are expressed as the means ± SEM (three replicates)
$^{*}$ statistically significant difference *vs.* F1S (control for F2S and F3S); $p<0.05$
$^{+}$ statistically significant difference *vs.* F1H (control for F2H and F3H); $p<0.05$
F1H – full water extract from herb
F2H – protein fraction of extract from herb
F3H – non-protein fraction of extract from herb
F1S – full water extract from seedlings
F2S – protein fraction of extract from seedlings
F3S – non-protein fractions of extract from seedlings

Statistical analysis of results (ANOVA) showed significant differences in the antioxidant activity of various concentrations of the CM extracts using the FRAP model ($F(4,10)=560.8$; $p=0.000$ for F1S; $F(4,10)=1531.1$, $p=0.000$ for F2S, $F(4,10)=1453.8$, $p=0.000$ for F3S and $F(4,10)=15203.7$, $p=0.000$ for F1H, $F(4,10)=25063.3$, $p=0.000$ for F2H, $F(4,10)=18084.8$, $p=0.000$ for F3H) (tab. 4). The antioxidant properties of the extracts was found to increase in

a dose-dependent manner. The trolox equivalent antioxidant capacity (TEAC) values varied from 25.92 to 99.65 $\mu$M/l (mean 58.69) for F1S, 33.23 to 90.84 $\mu$M/l (mean 58.65) for F2S, 31.38 to 116.63 $\mu$M/l (mean 72.26) for F3S, and 95.22 to 489.45 $\mu$M/l (mean 272.58) for F1H, 66.35 to 399.12 $\mu$M/l (mean 240.10) for F2H, 92.83 to 442,08 $\mu$M/l (mean 258.69) for F3H. According to calculation it was shown higher TEAC values in order F1H > F3H > F2H > F3S > F1S = F2S.

**Table 4.**

FRAP [$\mu$M/l] after the extracts from *Chelidonium majus* expressed as trolox equivalent

| Extract | FRAP [$\mu$M/l] | | | | | ANOVA |
|---|---|---|---|---|---|---|
| | 0.0125 [mg/0.1 ml] | 0.025 [mg/0.1 ml] | 0.050 [mg/0.1 ml] | 0.075 [mg/0.1 ml] | 0.10 [mg/0.1 ml] | |
| F1H | 95.22 ± 0.23 | 156.22 ± 1.11 | 286.64 ± 1.04 | 335.39 ± 1.90 | 489.45 ± 1.37 | $F(4, 10) = 15203.74$, $p=0.000$ |
| F2H | 66.35 ± 0.93$^+$ | 140.22 ± 1.26$^+$ | 238.32 ± 0.71$^+$ | 356.51± 0.42$^+$ | 399.12 ± 0.89$^+$ | $F(4, 10) = 25063.33$, $p=0.000$ |
| F3H | 92.83 ± 0.80 | 155.55 ± 1.48 | 253.84 ± 1.26$^+$ | 349.17 ± 0.66$^+$ | 442.08 ± 0.80$^+$ | $F(4, 10) = 18084.80$, $p=0.000$ |
| F1S | 25.92 ± 0.16 | 33.96± 0.36 | 57.66 ± 1.06 | 75.46 ± 0.94 | 99.65 ± 2.44 | $F(4, 10) = 560.81$, $p=0.000$ |
| F2S | 33.23 ± 0.97* | 38.12 ± 0.11 | 58.12 ± 0.88 | 73.07 ± 0.21 | 90.84 ± 0.33* | $F(4, 10) = 1531.12$, $p=0.000$ |
| F3S | 31.38 ± 0.07* | 47.79 ± 1.06* | 72.61 ± 1.58* | 92.91 ± 0.38 | 116.63 ± 0.47* | $F(4, 10) = 1453.80$, $p=0.000$ |

Data are expressed as the means ± SEM (three replicates)
* statistically significant difference *vs.* F1S (control for F2S and F3S); $p<0.05$
+ statistically significant difference *vs*. F1H (control for F2H and F3H); $p<0.05$
F1H – full water extract from herb
F2H – protein fraction of extract from herb
F3H – non-protein fraction of extract from herb
F1S – full water extract from seedlings
F2S – protein fraction of extract from seedlings
F3S – non-protein fractions of extract from seedlings

The analysis of our results clearly indicates that the scavenging capacity of the extracts, measured with all three methods (DPPH and ABTS radical scavenging capacities and a FRAP model), was found to increase in a dose-dependent manner. When measured by DPPH and FRAP method F1H demonstrated a higher antioxidant activity than F2H and F3H ($p<0.05$). Even more, in a FRAP assay the antioxidative activity was presented in the following way F1H > F3H > F2H > F3S > F1S = F2S ($p<0.05$). In ABTS assay such activity was higher in the case of F3H > F2H *vs.* extracts from seedlings (F3S > F2S) for all concentrations.

The our study is part of a trend of studies on the antioxidative properties of *Ch. majus* extracts or their fractions which suggest that a such antioxidant capacity may depend on flavonoid and/or total alkaloid contents [30–33]. The analysis of the current state of knowledge in the area of determination of the antioxidative potential of *Ch. majus* extract suggests that the most commonly used method of assessing the antioxidative capability was the DPPH method [26, 31–33], less frequently a FRAP method was used [30, 31]. Moreover, the different solvent were performed and the most frequently alcoholic (methanolic, ethanolic) or aqueous

extracts were analyzed. Also, acetone, ethyl acetate and petroleum ether *Ch. majus* extracts were analyzed in this field [26].

Then *et al.* [30] showed that the antioxidant activity (measured in a FRAP assay) of the alcoholic extracts (20 and 40%) of the *Ch. majus* herb may not be dependent on the alkaloid content during the vegetation period. Although the observed antioxidant activities of analyzed extracts increased dose-dependently (for the 20% (% alkaloid content: 0.172±0.008) and 40% (% alkaloid content: 0.380±0.009) alcoholic extracts antioxidant activities were 90.6±9.4 $\mu$M/l (=2.27±0.235 mg/0.1 ml) and 91.4±15.2 $\mu$M/l (=2.29±0.38 mg/0.1 ml), respectively), results from the FRAP assay for the herb material during the III, IV and VI month of vegetation period did not show a straight correlation [30]. These values are similar to values obtained in our analysis for the F1S and F2S fractions at the DPPH in the concentration of 0.05 mg/0.1 ml (tab. 2).

In other study Dragana Jakovljević *et al.* [26] showed the highest total flavonoid concentration in acetone and ethyl acetate extracts. In acetone extract a such content spanned between 135.37±1.47 RU/g in flowering stage to 291.58±1.11 RU/g in initial flowering stage, while in ethyl acetate extract the total flavonoid quantity was as follows: 190.60±1.74 – 248.23±0.97 RU/g for stage of rosette and initial flowering stage, respectively. The highest DPPH radical neutralizing abilities were obtained for the methanol extract of the plant during the rosette stage, which at a concentration of 50.72 mg/ml (=5.072 g/100 ml) neutralizes 50% of free radicals. Approximate activity was manifested in methanol extract of the stage before flowering ($IC_{50}$ value was 68.05 mg/ml (=6.805 g/100 ml). Our results values from DPPH radical scavenging capacities seems to be a bit more higher, especially in the case of F1H and F3H fractions.

## SUMMARY

In our study, three methods (FRAP assay, DPPH and ABTS radical scavenging assay) to determine the antioxidant capacity of various extracts from herb and seedlings of *Chelidonium majus* were employed. According to the results presented in this study, it seems that extracts from herb at all concentrations exhibited higher antioxidant capacities than extracts from the young seedlings. In two antioxidant tests (DPPH, FRAP) it was observed that full water extract from herb (F1H) exerted the highest antioxidant activity, but non protein fraction (F3H) and protein fraction (F2H) showed lower antioxidant activity (respectively). Moreover, in all tests a non-protein fraction from water extract from herb (F3H) was the most active, while the full water extract (F1S) and protein fraction (F2S) had less antioxidant activity. According to our best knowledge results presented in this manuscript concern the most complex evaluation of antioxidative potential of particular fractions of *Ch. majus* published so far, not only of the herb, but also seedling. Even more, the amount of some alkaloids,

possibly responsible for observed effects, were also determined for the first time in above mentioned fractions. The free radicals scavenging methodology was the most extensive.

Analysis of these results in regard to the previously cited ones, according to our point of view, allows to assume that at least in part, for the observed changes in antioxidant activity liable fractions of alkaloids, particularly of chelidonine and coptisine could be responsible, especially those presented in the greatest concentration in fraction F1H. The participation of other alkaloids in these activities should also be taken into consideration. It cannot be also excluded that the observed values of the variables studied between fractions from extracts of *Ch. majus* herb or seedlings may also be the result of specific interactions (interferences) with fraction of flavonoids and other chemical compounds, i.e. plant growth regulators in young seedlings. There is a need to conduct further research in this matter in order to verify our assumptions.

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*Conflict of interest: Authors declare no conflict of interest.*

## REFERENCES

1. WHO monographs on medicinal plants commonly used in the Newly Independent States. *Chelidonii herba*. World Health Organization. Geneva 2010:72-89.
2. EMA. European Medicines Agency. Committee on Herbal Medicinal Products (HMPC). EMA/HMPC/369801/2009. Assessment report on *Chelidonium majus* L., herba, 2011.
3. Migas P, Heyka M. Glistnik jaskółcze ziele (*Chelidonium majus* L.) we współczesnej terapii – wskazania i bezpieczeństwo stosowania. Postępy Fitoterapii 2011; 3:208-218.
4. Kędzia B, Łożykowska K, Gryszczyńska A. Skład chemiczny i zawartość substancji biologicznie aktywnych w *Chelidonium majus* L. Postępy Fitoterapii 2013; 3:174-181.
5. Mikołajczak PŁ, Kędzia B, Ożarowski M, Kujawski R, Bogacz A, Bartkowiak-Wieczorek J et al. Evaluation of anti-inflammatory and analgesic activities of extracts from herb of *Chelidonium majus* L. Centr Eur J Immunol 2015; 40(4):400-410. doi: http://dx.doi.org/10.5114/ceji.2015.54607
6. Arora D, Sharma A. A review on phytochemical and pharmacological potential of genus *Chelidonium*. Pharmacogn J 2013; 5:184-190. doi: http://dx.doi.org/10.1016/j.phcgj.2013.07.006
7. Gilca M, Gaman L, Panait E, Stoian I, Atanasiu V. *Chelidonium majus* – an integrative review: traditional knowledge versus modern findings. Forsch Komplementmed 2010; 17:241-248. doi: http://dx.doi.org/10.1159/000321397

8. Reuter S, Gupta S, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: How are they linked? Free Radic Biol Med 2010; 49(11):1603-1616. doi: http://dx.doi.org/10.1016/j.freeradbiomed.2010.09.006
9. Guedes RP, Bosco LD, Teixeira CM, Araujo ASR, Llesuy S, Bello-Klein A et al. Neuropathic pain modifies antioxidant activity in rat spinal cord. Neurochem Res 2006; 31:603-609. doi: http://dx.doi.org/10.1007/s11064-006-9058-2
10. Pandya CD, Howell KR, Pillai A. Antioxidants as potential therapeutics for neuropsychiatric disorders Prog Neuropsychopharmacol Biol Psychiatry 2013; 46:214-223. doi: http://dx.doi.org/10.1016/j.pnpbp.2012.10.017
11. Zhao B. Natural antioxidants protect neurons in Alzheimer's Disease and Parkinson's disease. Neurochem Res 2009; 34:630-638. http://dx.doi.org/10.1007/s11064-008-9900-9
12. Pham-Huy LA, He H, Pham-Huy C. Free Radicals, Antioxidants in disease and health. Int J Biomed Sci 2008; 4(2):89-96.
13. Powers SK, Jackson MJ. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiol Rev 2008;88(4):1243-1276. doi: http://dx.doi.org/10.1152/physrev.00031.2007
14. Valério DA, Georgetti SR, Magro DA, Casagrande R, Cunha TM, Vicentini FT et al. Quercetin reduces inflammatory pain: inhibition of oxidative stress and cytokine production. J Nat Prod 2009;72(11):1975-9. doi: http://dx.doi.org/10.1021/np900259y
15. Colomeu TC, Figueiredo D, Cazarin CB, Schumacher NS, Maróstica MR Jr, Meletti LM, et al. Antioxidant and anti-diabetic potential of *Passiflora alata* Curtis aqueous leaves extract in type 1 diabetes mellitus (NOD-mice). Int Immunopharmacol 2014; 18(1):106-15. doi: http://dx.doi.org/10.1016/j.intimp.2013.11.005
16. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease Oxid Med Cell Longev 2009; 2(5):270-278. doi: http://dx.doi.org/10.4161/oxim.2.5.9498
17. Tiong SH, Looi CY, Hazni H, Arya A, Paydar M, Wong WF et al. Antidiabetic and antioxidant properties of alkaloids from *Catharanthus roseus* (L.) G. Don. Molecules 2013; 18:9770-9784. doi: http://dx.doi.org/10.3390/molecules18089770
18. Tsoi B, Yi RN, Cao LF, Li SB, Tan RR, Chen M et al. Comparing antioxidant capacity of purine alkaloids: a new, efficient trio for screening and discovering potential antioxidants *in vitro* and *in vivo*. Food Chem 2015; 176:411-419. doi: http://dx.doi.org/10.1016/j.foodchem.2014.12.087
19. Sroka Z, Janiak M, Dryś A. Antiradical activity and amount of phenolic compounds in extracts obtained from some plant raw materials containing methylxanthine alkaloids. Herba Pol 2015; 61(3):53-66. doi: http://dx.doi.org/10.1515/hepo-2015-0022
20. Duda-Chodak A, Arko TT, Rus M. Antioxidant activity of selected herbal plants. Herba Pol 2009; 55(4):65-77.
21. European Pharmacopoeia. Monograph: greater celandine (*Chelidonii herba*). Sixth Edition, EDQM, Council of Europe, Strasbourg, 2011:1145-1146.
22. Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. Lebenson Wiss Technol 1995; 28:25-30.
23. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio Med 1999; 26(9/10):1231-1237.
24. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as measurement of „antioxidant power": The FRAP assay. Anal Biochem 1996; 239:70-76. doi:http://dx.doi.org/10.1006/abio.1996.0292
25. Stancic-Rotaru M, Mititelu M, Crasmaru M, Balaban D. Spectroanalytical profile of flavonoids from *Chelidonium majus* L. Roum Biotechnol Lett 2003; 8:1093-100.
26. Jakovljevic DZ, Stankovic MS, Topuzovic MD. Seasonal variability of *Chelidonium majus* L. secondary metabolites content and antioxidant activity. EXCLI J. 2013; 12:260–268.
27. Parvu M, Vlase L, Fodorpataki L, Parvu O, Rosca-Casian O, Bartha C, et al. Chemical composition of celandine (*Chelidonium majus* L.) extract and its effects on *Botrytis tulipae* (Lib.) lind fungus and the Tulip. Not Bot Horti Agrobot 2013; 41(2):414-426.
28. Kursinszki L., Sàrközi Á, Kéry Á, Szőke É. Improved RP-HPLC method for analysis of isoquinoline alkaloids in extracts of *Chelidonium majus*. Chromatographia 2006; 63:S131-S135.
29. Saravanan S, Arunachalam K, Parimelazhagan T. Antioxidant, analgesic, anti-inflammatory and antipyretic effects of polyphenols from *Passiflora subpeltata* leaves – a promising species of *Passiflora*. Ind Crop and Prod 2014; 54:272-280.

30. Then M, Szentmihályi K, Sárközi A, Szöllôsi Varga I. Examination on antioxidant activity in the greater celandine (*Chelidonium majus* L.) extracts by FRAP method. Acta Biol Szeged 2003; 47(1-4):115-117.
31. Stef DS, Gergen I, Trasca TI, Harmanescu M, Stef L, Biron R, et al. Total antioxidant and radical scavenging capacities for different medicinal herbs. Rom. Biotechnol Lett 2009; 14(5):4704-4709.
32. Hădărugă DI., Hădărugă NG. Antioxidant activity of *Chelidonium majus* L. extracts from the Banat county. J Agroalim Proc Technol 2009; 15(3):396-402.
33. Maji AK, Banerji P. *Chelidonium majus* L. (Greater celandine) – a review on its phytochemical and therapeutic perspectives. Int J Herbal Med 2015; 3(1):10-27.

## PORÓWNANIE DZIAŁANIA ANTYOKSYDACYJNEGO FRAKCJONOWANYCH WYCIĄGÓW Z SIEWEK I ZIELA *CHELIDONIUM MAJUS* L. PRZY UŻYCIU METOD DPPH, ABTS I FRAP

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Streszczenie

**Wstęp:** Nasze badania, będąc częścią trendu badawczego skupiającego się na ocenie aktywności antyoksydacyjnej ekstraktów i ich frakcji z *Chelidonium majus* wskazują, że działanie przeciwutleniające może być zależne od całkowitej zawartości flawonoidów i/lub alkaloidów. **Cel:** W badaniu skupiono się na ocenie aktywności antyoksydacyjnej ekstraktów z ziela dojrzałych roślin oraz siewek *Ch. majus* (pełny ekstrakt wodny, jego frakcja bezbiałkowa i frakcja białkowa). **Metody:** Całkowita zawartość flawonoidów oraz alkaloidów była oznaczana metodami spektrofotometrycznymi. Ilościową zawartość chelidoniny, koptyzyny, sanquinaryny, berberyny określono metodą HPLC-UV. Aktywność antyoksydacyjną oceniano przy użyciu (**1**) wolnego rodnika DPPH (2,2-difenylo-1-pikrylohydrazyl), (**2**) ABTS (kwas 2,2'–azynobis-(3-etylobenzotiazolino-6-sulfonowy)) oraz metodą (**3**) oznaczania zdolności redukowania jonów żelaza (FRAP). **Wyniki:** Wszystkie stężenia ekstraktów z ziela wykazywały większą pojemność antyoksydacyjną w porównaniu z ekstraktami z siewek. W dwóch testach antyoksydacyjnych (DPPH, FRAP) wykazano, że pełny ekstrakt wodny z ziela wywierał największą aktywność antyoksydacyjną, natomiast frakcje białkowa i bezbiałkowa tego ekstraktu wykazywały niższą aktywność. Pełny ekstrakt wodny z ziela zawierał najwyższe stężenie flawonoidów i alkaloidów w porównaniu z innymi analizowanymi próbkami. **Wnioski:** Wyniki badań sugerują, że szczególnie chelidonina i koptyzyna mogą być odpowiedzialne za obserwowane zmiany w aktywności antyoksydacyjnej z uwagi na to, że te alkaloidy oznaczono w największym stężeniu w pełnym ekstrakcie wodnym z ziela. Nie można również wykluczyć, że obserwowane różnice w wartościach badanych zmiennych pomiędzy ekstraktami z ziela *Ch. majus* i otrzymanymi z siewek mogą wynikać z zachodzenia interakcji flawonoidów z innymi związkami chemicznymi.

**Słowa kluczowe:** *Chelidonium majus, glistnik jaskółcze ziele, wyciąg ziołowy, aktywność antyoksydacyjna, DPPH, ABTS, FRAP, analiza fitochemiczna, alkaloidy, flawonoidy, analiza statystyczna*