

SYMPOSIUM

Examination on antioxidant activity in the greater celandine (*Chelidonium majus* L.) extracts by FRAP method⁺

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ABSTRACT Antioxidant activity in the alcoholic extracts (20 and 40%) of the greater celandine (*Chelidonium majus* L.) herb was investigated by FRAP (ferric reducing and antioxidant power) method. Since the antioxidant activity of the extracts greatly depends on the quality of compounds, the phytochemical examination for alkaloid- and element content were also examined. According to the results the antioxidant activity does not depend on the alkaloid content of the drug during the vegetation period and on the alkaloid content of the alcoholic extracts. It seems that the antioxidant activity of the extracts is also independent from the transition metal element content.

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KEY WORDS

Chelidonium majus L.
alcoholic extract
FRAP method
alkaloid content
metal ion content

The greater celandine (*Chelidonium majus* L.) is not listed in the Hungarian Pharmacopoeia in spite of the fact that the drug is commercially available in the herb-trade. In folk medicine the antiviral activity of the plant is attributed to its alkaloids present in the freshly outflowing latex (Dickson 1996). The alkaloid components of the orange coloured latex of the plant (chelidonine, chelerythrine, coptisine, sanguinarine, berberine etc.; Then et al. 2000) also have a number of beneficial effect, e.g. spasmolytic-, antiinflammatory-, antimicrobial-, antiviral-, antifungal-, antitumor activity and cytotoxic properties (Khayyal et al. 2001; Coon and Ernst 2002; Kokoska et al. 2002).

The antioxidant activity of the drug and extracts has not examined yet. Therefore, the antioxidant activity was measured by FRAP method and evaluated on the bases of alkaloid and element content. The the FRAP method means the ferric reducing ability of plasma or plants (Benzie and Strain 1996, 1999). Ferric to ferrous ion reduction at low pH causes a ferrous-tripyridyl-triazine complex which has absorbtion at 593 nm. FRAP values are obtained by comparing the absorbance changes at the given wavelenght and how influence the added plasma aliquots the FRAP values. Absorbance changes are linear over a wide concentration range with antioxidant mixture, including plasma or purified antioxidant mixture. The known antioxidants are interact very much. The FRAP assays are inexpensive, simple to prepare the reagents, the results are highly reproducibile and measurement takes no long time.

Materials and Methods

Fresh aerial parts of greater celandine (*Chelidonium majus* L.) were collected from the Botanical Garden of Budapest in 2002.

I. Extraction: The plant (5 g) was poured with alcoholic water (100 ml, 20 and 40%, 60°C) and allowed to stand at room temperature for 24 hours, then filtered.

II. Extraction for antioxidant activity of drug sample: The plant (1.5 g) was poured with double distilled water (200 ml) and allowed to stand at room temperature for 30 min, then filtered.

For the determination of total alkaloid content of plant and extracts, the reference method chosen was the measurement of chelidonine content according to the German Pharmacopoeia (DAB 10) as follows. The plant (0.75g) or the solution (25 ml) was extracted with CH₃COOH (200 ml, 12%, g/v) by refluxing on a water bath for 30 min. After cooling the solution was filtered into a volumetric flask (250 ml). The acetic acid extract (30 ml) was made alkaline (pH 8-9) with NH₄OH (25%) then extracted with CHCl₃ (3x30 ml) in a separatory funnel. The CHCl₃ phase was mixed and dried on anhydrous Na₂SO₄ and after filtration, CHCl₃ was evaporated under vacuum. The residue was redissolved in CH₃CH₂OH (2.5 ml) and transferred into a volumetric flask (25 ml). The trace residue was washed with diluted H₂SO₄ (10%, 3x5 ml) and also transferred into the volumetric flask, then the solution was diluted to 25 ml. A mixture of this solution (5 ml) and chromotropic acid (5 ml) was diluted with H₂SO₄ (98%) to 25 ml, then kept on a boiling water bath (100°C) for 10 min. After cooling, the absorption of the solution was measured at 570 nm against

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⁺In memory of Professor Béla Matkovics

Table 1. Alkaloid content (% g/100 g) and antioxidant activity ($\mu\text{mol/l}$) of the greater celandine (*Chelidonium majus* L.) plant herb during the vegetation period.

Harvested period	Alkaloid content (%)	Antioxidant activity ($\mu\text{mol/L}$)
III. month	0.218 ± 0.023	569.5 ± 9.41
IV. month	0.196 ± 0.015	289.5 ± 018.5
VI. month	0.117 ± 0.008	422.2 ± 32.1

Table 2. Alkaloid content (% g/100 ml) and antioxidant activity ($\mu\text{mol/l}$) in the alcoholic extracts of the greater celandine (*Chelidonium majus* L.).

Samples	Alkaloid content (%)	Antioxidant activity* ($\mu\text{mol/L}$)
20 % alcoholic extract	0.172 ± 0.008	90.6 ± 9.4
40 % alcoholic extract	0.380 ± 0.009	91.4 ± 15.2

*20 % and 40 % alcoholic-aqueous solution have no antioxidant activity.

Table 3. Element concentration ($\mu\text{g/ml}$) in the alcoholic extracts of the greater celandine (*Chelidonium majus* L.).

Elements	0 % alcoholic extract	0 % alcoholic extract
Ca	10.94 ± 0.29	6.25 ± 0.20
Cu	0.273 ± 0.173	0.188 ± 0.074
Fe	0.217 ± 0.023	0.137 ± 0.029
K	52.36 ± 5.07	67.94 ± 0.17
Mg	17.01 ± 0.63	11.90 ± 0.12
Mn	0.141 ± 0.009	0.086 ± 0.008
Na	39.21 ± 0.80	23.50 ± 0.20
P	30.41 ± 0.27	19.83 ± 0.15
Zn	0.464 ± 0.006	0.346 ± 0.006

the blank solution. The extinction coefficient in 1% solution in a 1 cm vessel was 933 ($100 \text{ ml g}^{-1} \text{ cm}^{-1}$).

The FRAP method for measuring the ferric reducing ability of plasma (FRAP) or plants (Benzie and Strain 1996, 1999) is the following:

Reagents:

1. Acetate buffer, 300 mmol/L pH 3.6 (3.1 g sodium acetate $\times 3\text{H}_2\text{O}$ and 16 ml acetic acid in 1000 ml buffer solution).

2. 10 mmol/l 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mmol/l HCl.

3. 20 mmol/l $\text{FeCl}_3 \times 6\text{H}_2\text{O}$ in distilled water.

FRAP working solution: 25 ml acetate buffer (1), 2.5 ml TPTZ solution and 2.5 ml $\text{FeCl}_3 \times 6\text{H}_2\text{O}$ solution. The working solution must be always freshly prepare.

Aqueous solution of known $\text{FeSO}_4 \times 7\text{H}_2\text{O}$ was used for calibration.

Assay: Blank: FRAP reagent.

Sample: FRAP reagent 1,5 ml and sample solution 50 μl .

Monitoring up to 5 min at 593 nm, 1 cm light path and 37°C. Fe(II) standard solution tested in parallel. Calculation: using the calibration curve.

Element concentration of samples was determined by an inductively coupled plasma atomic emission spectrometer (ICP-AES). Type of instrument: Atom Scan 25 (Thermo Jarrell Ash), a sequential emission spectrometer. Sample handling: the samples (50 ml of evaporated extract) were digested with a mixture of HNO_3 (5 ml) and H_2O_2 (3 ml) in teflon vessels. After digestion the samples were diluted to 25 ml, from which the following elements were determined in three parallel measurements: Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn.

Results and Discussion

The drug samples was collected during the vegetation period in different time to observe the changes of the antioxidant activity with total alkaloid content. Total alkaloid content of the samples was measured accordance with the German Pharmacopoeia (DAB 10), and the antioxidant activity was assayed by FRAP method (Table 1). According to the results we stated that the alkaloid content of the drug changes during the vegetation period and it seems that the antioxidant activity does not depend on the alkaloid content of the drug.

Alcoholic extracts (20 and 40%) of the greater celandine contains different amount of alkaloids with almost the same and very low antioxidant activity (Table 2). This result confirms our previous measurements obtained during vegetation period. Alcoholic solutions (20 and 40%) do not have an influence on the antioxidant activity.

The extracts contain elements as well dissolved in or bound by organic compounds (Buzuk et al. 2001). Element content of the extracts is very low (Table 3) and they do not contain transition metal ions in higher concentration than other alcoholic extracts (Szentmihályi and Then 2001; Szentmihályi et al. 2001).

Though the greater celandine (aqueous extract) has antioxidant activity, this value seems to be independent from its alkaloid and element content.

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