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Effect of sample handling on alkaloid and mineral content of aqueous extracts of greater celandine (*Chelidonium majus* L.)

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Abstract

The authors examined the extraction of alkaloids from the greater celandine (*Chelidonium majus* L.) by different methods (traditional pressing and tea making, microwave and supercritical fluid extraction). The extractants were water and propylene glycol. For comparison of the extraction methods, the yield was evaluated according to total alkaloid content measured by spectroscopy. The highest alkaloid yield was obtained by microwave extraction and by making tea. Distribution of the components was studied by thin-layer chromatography and densitometry. The concentration and the ratio of alkaloid components in extracts are significantly different depending on the extraction method. The solution obtained by supercritical fluid extraction contains coptisine and chelidonine, while berberine could be obtained by microwave extraction only. Extracts with high coptisine content were obtained by supercritical fluid extraction, followed by pressing and microwave extraction. Mineral element content of the drug and extracts was also determined by inductively coupled plasma atomic emission spectrometry. Element content (Na, Ca, Fe) was found to be highest in microwave extracts.

Keywords: *Chelidonium majus*; Tea; Food analysis; Extraction methods; Alkaloids; Coptisine; Metal cations; Chelidonine

1. Introduction

The greater celandine (*Chelidonium majus* L.) belongs to the Papeveraceae family. As a specific feature of the family the orange coloured latex of the plant contains alkaloids. The greater celandine is not listed in the Hungarian Pharmacopoeia, or in most other Pharmacopoeias in spite of the fact that the drug is commercially available in the herb-trade.

The most effective alkaloid components of the plant (chelidonine, chelerythrine, coptisine, sanguinarine, berberine etc. Fig. 1) [1–3] have spasmolytic, anti-inflammatory, antimicrobial, antiviral, antifungal and antitumor activities and cytotoxic properties [4–8].

In folk medicine the antiviral activity of the plant is attributed to the alkaloids present in the freshly outflowing latex. During drying the plant loses 4/5ths of its original mass and most of its effective compounds, which are probably responsible for the antiviral activity of killing warts. On the other hand, the drug retains its microbiological effect and is, therefore, considered an important substance applied for dental purposes [9].

The most widely applied method of utilizing medicinal drugs is making tea, sometimes, however, other extraction techniques are also used. Since the effect of sample preparation on the alkaloid content of extracts has not been examined so far, therefore,

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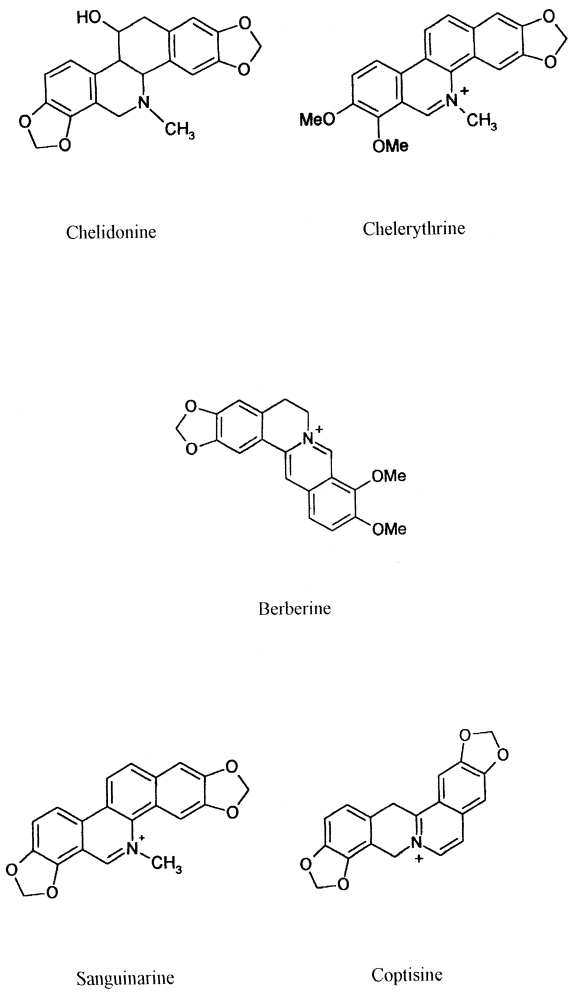

Fig. 1. Main alkaloid compounds of greater celandine.

we carried out novel sample preparation methods (supercritical fluid and microwave extraction) to obtain extracts rich in alkaloids from *Chelidonium majus*. Comparison of the extracts was based on total alkaloid and coptisine content, since a German working group found that in many samples coptisine was the main alkaloid in the herb of greater celandine [10]. The extracts also contain elements dissolved in or bound by organic compounds, another purpose of these experiments was to examine the effect of different sample handling methods on the element content of extracts.

2. Experimental

2.1. Materials

Fresh aerial parts of greater celandine (*Chelidonium majus* L.) were collected from the Botanical Garden of Budapest in 1998.

2.2. Preparation of extracts

For tea, the plant (5 g) was poured with deionized water (100 ml, 60°C) and allowed to stand at room temperature for 24 h, then filtered.

Pressing with water was carried out as follows: The plant (5 g) was allowed to stand in deionized water (100 ml) for 24 h, then the aqueous latex was pressed with a pressing machine.

For pressing, the plant (50 g) was pressed with a pressing machine.

Supercritical fluid extraction (SFE) was carried out in a high pressure, flow-up stream extraction apparatus (University of Veszprém). The solvent was technical-grade CO_2 obtained from Repcelak Gas Trade (Hungary) and propylene glycol was purchased from Merck. The flow-rate (1.0–1.5 l min^{-1}) was ensured with the use of membrane pump (LEVA EL-1 Tip, Germany) and measured with a gas meter. The fresh plant (25 g) was extracted with carbon dioxide (46 g) at 38°C and 250 bar. The parameters were not optimised for alkaloid yield. Alkaloids of greater celandine are very sensitive, therefore relatively low temperature and high pressure was chosen for the extraction. Cumulative fractions were weighed up to equilibrium solubility. The CO_2-rich fluid was removed from the extractor through a heated needle valve and expanded to atmospheric pressure. The extract was received in water (90 g) in a cooled container, allowed to stand for 15 min to reach room temperature in order to facilitate CO_2 release. The weight constant of the extract was then determined. Carbon dioxide is not a good solvent for alkaloids, the first supercritical extraction, however, was made from fresh plant with relatively high water content, which may act as a solvent modifier. The residue was again extracted with carbon dioxide and propylene glycol (65 g) as a modifier. Details on the apparatus and methods were reported previously [11]. Part of the initial propylene glycol remained in

the extraction column, which caused some solvent loss.

Microwave extraction was carried out in a MarsX apparatus. The plant (2.5 g) was extracted in deionized water (50 ml) at 40° and 65°C. Extraction time was optimized for total alkaloid yield. Maximum alkaloid content was obtained at 40°C with 25 min extraction, and at 65°C in a 20 min extraction period.

2.3. Measurements of compounds

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 (0.75 g) or the solution (25 ml) was extracted with acetic acid (200 ml, 12%, w/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 NH_4OH (25%) then extracted with $CHCl_3$ (3×30 ml) in a separatory funnel. The chloroform phase was mixed and dried on anhydrous Na_2SO_4 and after filtration, chloroform was evaporated under vacuum. The residue was redissolved in ethanol (2.5 ml) and transferred into a volumetric flask (25 ml). The trace residue was washed with dilute sulfuric acid (10%, 3×5 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 sulfuric acid (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 the blank solution. The extinction coefficient in 1% solution in a 1 cm vessel was 933 (100 ml g^{-1} cm^{-1}).

Separation and measurement of alkaloids in the extracts were performed by thin-layer chromatography. The extract was dissolved in acetic acid (12%, w/v), the solution was refluxed on a water bath for 30 min and after cooling the solution was filtered. The acetic acid solution of the extract was poured into a separatory funnel, then NH_4OH (25%) was carefully added to the solution up to pH 9. The solution was extracted with $CHCl_3$ and the organic solution was dried on anhydrous Na_2SO_4, and $CHCl_3$ was evaporated under vacuum. The residue was redissolved in $CHCl_3$ (2 ml) from which 5 μl was used for chromatographic measurements. Silica gel (GF_{254}, Merck) and an eluent, 1-propanol–formic acid–water (90:1:9) was applied, as recommended by Wagner et al. [12]. The standard was a 0.1% chloroformic solution of berberine (Merck). For quantitative examination, the extent of the spot was measured with a densitometer at 365 nm (Shimadzu 169).

The element concentration of samples was determined by inductively coupled plasma atomic emission spectrometry (ICP–AES). An Atom Scan 25 (Thermo Jarrell Ash), a sequential emission spectrometer was used. The samples (0.5 g plant or 50 ml of evaporated extract) were digested with a mixture of HNO_3 (5 ml) and H_2O_2 (3 ml) in PTFE vessels. After digestion the samples were diluted to 25 ml, from which the following elements were determined in three parallel measurements: B, Ca, Cr, Cu, Fe, K, Li, Mg, Mn, Na, S, Zn.

2.4. Statistical analysis

Mean values and standard deviation (SD) were calculated from the results. One way analysis of variance was applied for comparison of the mean values.

3. Results and discussion

One hundred milliliters of extract was obtained from the plant by tea making, pressing with deionized water and by microwave extractions. The yield was 6.19 g latex by pressing from 50 g of the fresh plant, 94.8 g aqueous solution of supercritical fluid extraction, while supercritical fluid extraction of the residue with propylene glycol yielded 37.22 g extract.

The extracts dissolve significantly different amounts of substances (Table 1). The highest dry matter content was obtained from the pressed latex, followed by aqueous solution of pressed latex and then by supercritical fluid extraction with propylene glycol. The solution obtained by microwave extraction had a surprisingly low amount of dry material.

The total alkaloid content of the extracts was

Table 1
Dry matter content ($n=3$, except for supercritical fluid extraction, where $n=1$), total alkaloid yield (in chelidonine %, measured by DAB 10, $n=3$) and coptisine content (%) in the extracts of greater celandine referred to berberine content

Extract	Dry matter content of extract (g/100 g plant)	Total alkaloid yield (g chelidonine/100 g plant)	Coptisine content (mg/100 ml)
Tea	1.140±0.014	0.59±0.03	0.654±0.022
Pressed latex	2.893±0.056	0.21±0.02	0.683±0.026
Pressed latex with deionized water	1.389±0.023	0.19±0.02	6.62±0.31
Supercritical fluid extract with water	1.087	0.26±0.02	0.698±0.019
Supercritical fluid extract with propylene glycol	1.330	0.07±0.008	0.759±0.028
Microwave extract at 40°C	0.674±0.011	0.37±0.03	0.664±0.013
Microwave extract at 65°C	0.676±0.009	0.61±0.04	0.660±0.038

measured by spectrophotometry according to the German Pharmacopoeia (DAB 10), and the results refer to the starting material (Table 1). The total alkaloid yields obtained from the plant by different methods differ significantly ($P<0.0001$). The highest alkaloid yield was obtained in the case of microwave extraction at 65°C (0.61 g alkaloid from 100 g plant) and tea (0.59 g alkaloid from 100 g plant), while the worst results were obtained by pressing (0.19 g and 0.21 g alkaloid from 100 g plant). Supercritical fluid extraction with water and propylene glycol was actually one extraction, as the residue of the first extraction was extracted with CO_2 and propylene glycol.

Since the amount of the individual components is important, the alkaloids in the extracts were measured by thin layer chromatography. Separation of the components was achieved with the eluent recommended by Wagner et al. [12]. The alkaloids were identified by standard addition in the case of chelidonine, chelerythrine, sanguinarine and berberine. In our samples coptisine was the main alkaloid as described by others for the plant of greater celandine [10]. As we had no access to coptisine, coptisine was identified according to relative retentions compared to those of the other alkaloids and comparison with literature data [12]. The amount of coptisine was measured at the level of $R_f=0.15$ by densitometry and the results were referred to berberine ($R_f=0.36$) content and summarized in Table 1. The densitogram in Fig. 2 shows the coptisine peaks of extracts measured at the level of the retention factor 0.15. The coptisine content of the different solutions are almost the same (except for pressed latex), although differ statistically ($P<0.05$). The difference in the total alkaloid content of

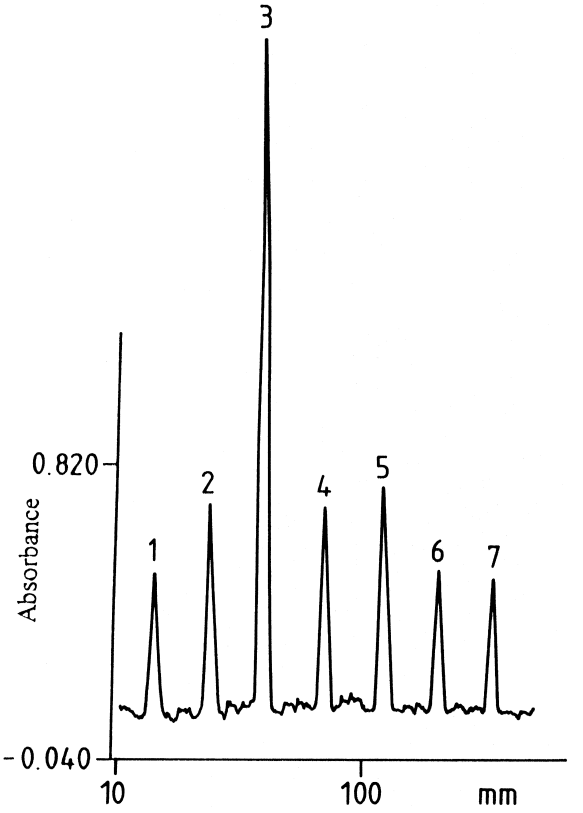

Fig. 2. Densitogram of greater celandine extracts obtained by different sample handling at the level of coptisine peak ($R_f=0.15$) 1=Tea, 2=pressed latex with deionized water, 3=pressed latex, 4=supercritical fluid extract in water, 5=supercritical fluid extract with propylene glycol, 6=microwave extract at 40°C, 7=microwave extract at 60°C. The densitograms represent the same amount of initial plant except for pressed latex (3), where the initial plant was ten times as high.

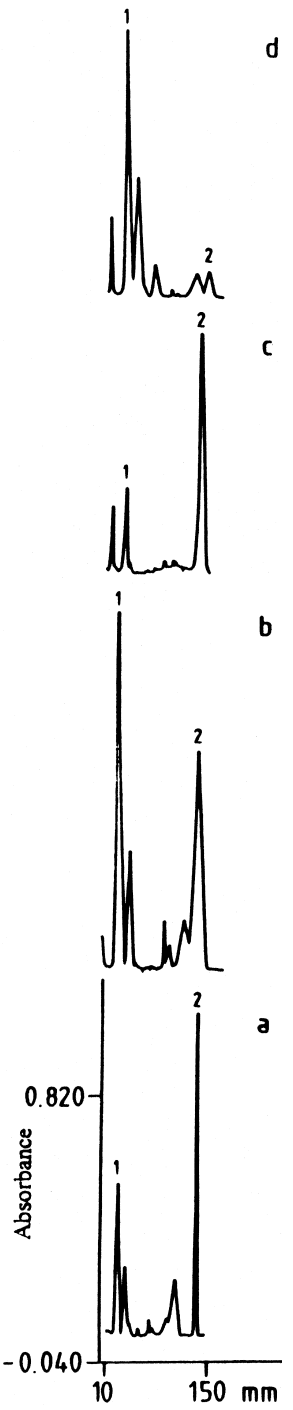

Fig. 3. Densitograms of greater celandine extracts obtained by different sample handling (a) tea, (b) pressed latex, (c) supercritical fluid extract with water and (d) microwave extract at 40°C. Peak numbering: 1=coptisine, 2= chelidonine.

extracts, expressed in absolute values and statistically, are higher than the differences in the coptisine content of the extracts. This suggests that the concentration of the other alkaloids significantly changes according to the extraction methods applied. For example, the coptisine content of the extract obtained by supercritical fluid extraction is almost equal to that of the solutions obtained by the other extraction techniques, but the supercritical fluid extract results in a rare alkaloid composition, containing only coptisine and chelidonine, while the microwave extracts contain several different alkaloids. The microwave extract is the only solution which shows berberine content (Fig. 3), while the chelidonine (R_f=0.8) content of the solution is lower than that of the pressed extracts.

In our assumption, the elements greatly contribute to the therapeutic effect of the plant since they are also present in the extracts. For example, the diuretic effect of the plant may also depend on the potassium–sodium ratio in teas [13]. Element concentrations of the plant and extracts measured by ICP–AES are given in Table 2. The plant contains average element concentration [14]. The dissolved mineral element content of the extracts is not high in view of their absolute values (except for potassium) although it is important that these elements are present both in the aqueous extracts and the pressed latex. In the case of supercritical fluid extraction the residue was measured and the amount of dissolution was calculated. As can be seen the dissolution percentage is relatively high, therefore, these results have not been evaluated. About half the amount of potassium and phosphorus, nearly forty percent of magnesium, and more than twenty percent of boron, manganese and zinc is dissolved into the tea together with the organic compounds. In the case of microwave extraction, the dissolution of elements shows lower values. According to these results element dissolution seems to be time dependent. On average, hardly 2–3% of the elements dissolved into the pressed latex (magnesium 5%, potassium 4.45%, calcium 3.56%, sodium 2.48%, sulfur 2.48%, zinc 1.34%, chromium 0.42% and copper 0.56%).

4. Conclusion

The total alkaloid content and alkaloid compo-

Table 2
Element content in greater celandine and extracts

Elements	Plant (mg/kg dry matter ±SD, $n=3$)	Tea (mg/l) and dissolution in parenthesis (%)	Pressed latex (mg/kg) and dissolution in parenthesis (%)	Microwave extract at 40°C (mg/l) and dissolution in parenthesis (%)	Microwave extract at 60°C (mg/l) and dissolution in parenthesis (%)	Residue of SFE (mg/kg±SD, $n=3$) and dissolution in SF extract in parenthesis (%)
B	17.36±0.24	0.260 (29.97)	2.45 (2.46)	0.181 (20.91)	0.104 (11.98)	13.33±0.42 (38.57)
Ca	10573±235	45.73 (8.65)	2089 (3.56)	67.85 (12.83)	50.45 (9.54)	6607±121 (50.01)
Cr	0.472±0.018	0.001 (3.95)	0.042 (0.42)	<0.02	<0.02	<0.2
Cu	18.57±0.39	0.290 (31.23)	0.275 (0.56)	0.067 (7.22)	0.054 (5.82)	19.55±0.51 (15.78)
Fe	196.9±2.9	0.197 (2.00)	3.06 (0.28)	0.379 (3.84)	0.454 (4.61)	87.42±1.07 (64.48)
K	32915±670	885.4 (53.8)	7253 (4.49)	262.5 (15.95)	249.2 (15.14)	12764±136 (68.37)
Mg	1739±17	33.88 (38.96)	432.7 (5.00)	13.31 (15.30)	10.85 (12.48)	794.6 ±13.0 (63.45)
Mn	21.39±5.0	0.227 (21.26)	2.56 (0.15)	0.114 (10.66)	0.080 (7.48)	10.38±0.07 (61.18)
Na	203.9±5.10	0.194 (1.89)	2.71 (2.48)	5.21 (51.15)	7.18 (70.38)	245.8±9.3 (3.56)
P	4383±87	124 (56.64)	630.5 (6.68)	25.62 (11.69)	20.97 (9.57)	1807±89 (67.02)
S	2833±44	41.72 (29.45)	160.0 (2.15)	6.25 (4.41)	4.21 (2.97)	1342±45 (62.10)
Zn	45.54±1.37	0.53 (23.25)	3.06 (1.34)	0.256 (11.22)	0.246 (10.78)	32.20±0.67 (43.43)

nents of the extract is greatly dependent on sample handling. The highest total alkaloid yield was obtained for microwave extraction, while supercritical fluid extraction gave the highest coptisine content. The alkaloid composition of tea and pressed latex was similar, while the two major components were present only in the supercritical fluid extract. Berberine was absent from most solutions except for the microwave extract. The aqueous extracts of greater celandine contain macro and micro elements as well, the concentration of which also depends on the method of extraction.

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