ELEMENTS, ALKALOIDS AND ANTIOXIDANT VALUE OF *CHELIDONIUM MAJUS* L. AND THE EXTRACTS OBTAINED BY DIFFERENT EXTRACTION METHODS

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The active components of *Chelidonium majus* (greater celandine) is based on its sensitive and effective biologically active agents. The active components of greater celandine extracts and, therefore, their usability depends on the extraction methods. This project was to evaluate and compare the components in the extracts obtained by different new and traditional extraction methods. The extracts were obtained by aqueous and alcoholic extraction, supercritical fluid extraction, pressing-centrifugation method, microwave extraction and were examined for alkaloids, elements, and antioxidant activity. The rhizome has the highest tannin, polyphenol, and alkaloid content, while aerial parts of the plant show the highest flavonoid contents and antioxidant activities. The extracts also contain metal ions contributing to the favourable therapeutic effects that can be mainly Cu, Fe, Mn, Cr, and Zn. The traditional pressed extracts rich in alkaloids confirm their usage for the treatment of warts and these extracts also contain Cu and Fe in concentrations that are effective against viruses.

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INTRODUCTION

Greater celandine (*Chelidonium majus* L., Figure 1) is a member of the Papaveraceae family. Alkaloid rich orange-colored latex flows out from broken stems and roots. The dried latex is available in the herb-trade or its products in pharmacies for internal and external use and in cosmetic shops for external use.[1] This proves the increasing use of the natural materials in therapy.

Figure 1. Rhizome and flowering plant of *Chelidonium majus* L.

Greater celandine is found in the vicinity of human habitations as a weed. It grows wild in the whole of Europe and almost everywhere in Asia and today, even in North America as well. Besides effective bioactive alkaloid components (coptisine, chelidonine, chelerythrine, sanguinarine, berberine, protropine, etc.), greater celandine contains flavonoids, chelidonine acid, resin, fruit acids (malic acid, citric acid, tartaric acid), vitamin C, volatile oil and metals as well.[2,3] The main use of greater celandine is externally against warts and corns, internally for healing liver and gallbladder diseases.[4] Bioactive components of the plant extracts have wide range effects as they have antioxidant, spasmolytic, anti-inflammatory, antimicrobial, antiviral, antifungal, antitumor activity and cytotoxic properties.[5-9]

Folk medicine indicates the antiviral activity of fresh plants and mainly to its alkaloids in freshly flowing out latex, which is used externally.[10,11] But after drying it, the latex loses its effective compound, which is responsible for the killing of warts. Other microbiological effects of the latex still remain that is why it becomes an important effective substance of the tooth-paste and the mouth-wash as well.[12,13]

For internal use of the medicinal drugs, the most often applied method is the tea making against gall bladder and liver problems. Nevertheless, other extraction methods are also applied, e.g., tinture. In the case of greater celandine pressing method for obtaining fresh latex with sensitive quaternary nitrogen agent content is the traditional extraction. Since the main bioactive components of greater celandine can change and their concentrations decrease with time, we examined different technological methods to study and get extracts of same or similar bioactive agent content as present in fresh plant or pressed latex. The metal elements are also found in the plant and extracts. These elements supposed to contribute to the favorable therapeutic effect. Therefore, the metal ion concentration in the drug and some extracts were measured as well to detect the soluble element. Antioxidant activity is connected to the polyphenolic compounds such as flavonoids.[14] Although there is no positive correlation between them in all cases, the higher antioxidant activity always implies a higher content of antioxidant type compound.

The primary objective was to study the *C. majus* L latex and different extracts for organic and inorganic agents as well as for antioxidant activity. A further objective was to compare and evaluate the measured parameters in different parts of the plant and extracts critically in the view of the main indication field.

MATERIALS AND METHODS

The samples studied were greater celandine plants freshly collected from the Botanical Garden of Budapest in the flowering state in May. After identification of the plant, a sample was placed in the plant warehouse of the Institute of Pharmacognosy (213/07). All kinds of measurement and extract were made from the fresh plant right away after collection except the element content determination for which the samples were dried (Sample numbers: 778-780).

For the supercritical fluid extraction (SFE), the solvent was technical grade carbon dioxide obtained from Répcelak Gas Trade (Hungary). Alcohol (96 %), propylene glycol solvents, nitric acid (37 %), hydrogen peroxide (30 %) were high purity grade and were purchased from Reanal Ltd (Budapest, Hungary). Chelidonine was purchased from Merck Ltd (Germany) while berberine obtained from Sigma-Aldrich (Hungary). High purity water (18.3 MΩ.cm) was made by Millipore equipment (Merck Ltd).

ICP multi-element standards (CPAchem Ltd; Stara Zagora, Bulgaria) were used as standard solutions.

Preparation of extracts

The aqueous extract was made from 6 g plant sample with 100 mL of deionized boiling water. After standing 24 h, it was filtered.

For the pressed method, a fresh plant (100 g) was pressed with a pressing machine obtaining 17.6 g latex (Sample number: SB.CH-12/5).

For the pressed with water method, fresh plant (53.28 g) was pressed with seizing fluid of water and centrifuged, obtaining 37.52 g filtrate (Sample number: SB.H-12/6).

Alcoholic extract was made from 5 g sample with 100 mL of alcohol (96 %). The suspension was kept for 3 days. Then it was filtered (Sample number: TM.CH-A/96).

SFE with water, propylene glycol and alcohol was made in a high-pressure flow-up stream extraction system (University of Veszprém) similarly, as was published earlier. Nevertheless, some parameters (e.g., temperature) and plant to solvent ratio are different.[15,16] SFE with water was made from 62.5 g fresh wet plant with 237 g of carbon dioxide at 35 ^{o}C and 250 bar. The extract (6.5882 g, 10.54 %) was received in 100 mL of water (Sample number: IV-62). SFE with propylene glycol was prepared from 97.65 g plant with 198 g carbon dioxide in the first step obtaining 6.55 g extract (6.71 %). At the second step, the plant residue was extracted once more with 280 g carbon dioxide and 51.50 g propylene glycol. The cumulated extract was 25.99 g solution (Sample number: IV-63). SFE with alcohol (96 %) was prepared from 31.48 g plant with 290 g of carbon dioxide in the first step. The extract yield was 1.8478 g (5.87 %). After this, the residue was extracted again with 96 g carbon dioxide and 10.90 g alcohol. The cumulated extract was 8.59 g solution (Sample number: IV-61).

Microwave extraction was carried out in a MarsX apparatus. The fresh plant (1 g) was extracted in 10 mL of deionized water at 100 ^{o}C for 3x3 min. with 500 W (Sample number: MW.CH-32).

The amount of fresh plant used for different extraction methods, and the gained extracts are summarized in Table 1. The extracts are stable for a long time in the refrigerator.

Table 1. Amount of initial greater celandine and the gained extracts.

Type of extraction	Amount of initial plant material (g)	Gained extract
Tea	6	100 mL
Pressed latex	100	17.6 g
Pressed latex with water	53.28	37.52 g
Alcoholic (96 %)	5	100 mL
SFE with water	62.5	100 mL
SFE with propylene glycol	97.65	25.99 g
SFE with alcohol	31.48	1.85 g
Microwave with water	1	10 mL

Measurements of organic compounds

Total flavonoid content was measured by spectrophotometry at 420 nm according to the German Pharmacopoeia (DAB10), after formation aluminum complex .[17,18]

The polyphenol content was measured by spectrophotometry according to the Singleton and Rossi method.[19] The absorbance of the samples was read at 760 nm with a Hitachi U-2000 spectrophotometer. Three parallel measurements of each sample were made against a blank that was prepared under the same conditions as the samples. Gallic acid was used as a reference solution, and the results were expressed in gallic acid equivalent. Tannic acid content was determined according to the description of Hungarian Pharmacopoeia (Ph.Hg.VIII.).[20]

Total alkaloid content was determined by spectrometry at 570 nm after complex formation with chromotropic acid according to the guideline of the German Pharmacopoeia (DAB10), and it was expressed in chelidonine content.[18] This method is official in the Hungarian Pharmacopoeia and the European Pharmacopoeia (Ph. Eur. 5) as well.[20,21]

Alkaloid components from the samples were determined by thin-layer chromatographic techniques with densitometry after extraction with methanol since this is an appropriate method for celandrine alkaloids nowadays as well.[22] Separation of coptisine and berberine from the other alkaloids, then the measurement of the samples was performed on precoated silicagel (Kieselgel60 F254).

Table 2. Bioactive agent content and ± standard deviation (%, g 100 g^{-1}, n=3) in the plant parts of fresh greater celandine.

Part	Flavonoid	Tannin	Polyphenol	Alkaloid
Rhizome	0.120±0.008	9.21±0.12	15.90±0.96	1.98±0.020
Stem	0.278±0.005	6.25±0.21	11.99±0.58	0.582±0.110
Leaf	0.404±0.008	6.54±0.16	13.38±0.45	0.691±0.025
Herb	0.392±0.004	6.26±0.27	11.71±0.39	1.85 ±0.02

Table 3. Amount of alkaloid components with ± standard deviation (%, g 100 g^{-1}, *n*=3) in the plant parts of fresh greater celandine.

Part	Berberine, %	Coptisine, %	Chelidonine, %
Rhizome	0.024±0.004	0.072±0.004	1.28±0.02
Stem	0.012±0.002	0.063±0.003	0.45±0.03
Leaf	0.065±0.004	0.021±.0.001	0.52±0.03
Herb	0.0024±0.0003	0.023±0.010	0.44±0.02

Eluent was 1-propanol, formic acid and water (90:1:9) recommended by Wagner and Bladt.[23] The standard was 1 % solution of chelidonine and berberine. For the quantitative examination, the extent of the spot was measured by the densitometer (Shimadzu 169). Other alkaloid content (coptisine) was expressed in chelidonine content.

Measurements of inorganic elements

The concentration of elements in samples was determined by inductively coupled plasma optical emission spectrometry (ICP-OES). The instrument was Spectro Genesis ICP-OES (Kleve, Germany). The plant samples were digested in an open digestion system with a mixture of HNO_3 (5 mL) and H_2O_2 (2 mL) then made up to 25 mL with deionized water.[24] In the case of extracts, the solvents were evaporated first, then it was digested as the plant samples. Three parallel solutions were made from each sample and the measurements for the micro- and macroelement (Al, B, Ba, Ca, Cd, Cr, Co, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Ti, V, Zn) were performed three times. The elements whose concentrations were below the detection limit in all samples were omitted from the tables.

Antioxidant value by FRAP method

The FRAP (the ferric reducing ability of plants) measurement was carried out by the following modified Benzie and Strain method.[25] Blank is FRAP reagent, the sample is 1.5 mL FRAP reagent and 50 µL sample solution then monitoring at 593 nm up to 5 min in 1 cm light path at 37 ºC. An aqueous solution of known $FeSO_4.7H_2O$ was used for calibration, and the result was calculated according to the calibration curve.[26] For measuring of the samples, extractions were made from 1.5 g powdered dry latex with 200 mL of water. After standing at room temperature for 30 min, it was filtered than measured with FRAP reagent.[27,28] The extracts were measured with FRAP reagent. The antioxidant values of samples expressed in µmol L^{-1}, since they refer to the solutions made.

FRAP reagent: The reagent solution contains 25 mL acetate buffer (300 mmol L^{-1}, pH 3.6; 3.1 g sodium acetate trihydrate and 16 mL acetic acid in 1000 mL distilled water), 2.5 mL TPTZ solution (10 mmol/l 2,4,6-tripyridyl-S-triazine in 40 mmol L^{-1} HCl) and 2.5 mL $FeCl_3.6H_2O$ solution (20 mmol L^{-1} $FeCl_3.6H_2O$ in distilled water).

Statistical analyses

The calculations of means and standard deviation as well as the statistical analysis were performed using Microsoft Office Excel 2016 and Statistica 7 (StatSoft Inc., Tulsa. USA) software. A significant difference was set at $P<0.05$.

RESULTS AND DISCUSSION

Organic agents of greater celandine drug

According to the officially accepted spectroscopic determination of the total active substance content, greater celandine has significant active substance content (Table 2). Greater celandine is a plant with valuable active ingredients, flavonoids, alkaloids, polyphenols, and tannins. The tannin, polyphenol, and alkaloid content of the rhizome is particularly outstanding, which is significantly ($P<0.05$) higher than that of the other parts of the plant. Flavonoids, on the other hand, were found to be significantly higher ($P<0.05$) amounts in the leaf and herb, although they were not in extremely high concentrations (Table 2). The highest alkaloid content is accumulated in the rhizome, but it is also significant in the other parts of the plant. Although the plant is of alkaloid content mainly, the tannin and polyphenol contents in all parts are outstanding also and according to the correlation calculation, the tannin content (Table 2) is a significant positive correlation with the polyphenol content (R=0.955, $P<0.05$). The active agents in the plant and plant parts of greater celandine are in good accordance with the data in the literature,[29,30] and the highest alkaloid content was measured in the rhizome that is not surprising.

The amount of alkaloid components was determined by HPLC and densitometry. The results showed that chelidonine is the major alkaloid component, whereas coptisine is present in smaller amounts (Table 3). The alkaloid composition also varies according to the plant parts, but the main alkaloid chelidonine is present in all parts of the plant. The amount of alkaloid components was correlated well with the results obtained by HPLC[31] and is in good agreement with earlier data in the literature.[32]

Elements in greater celandine drug

The element content in the parts of greater celandine is represented in Table 4. The concentration of most elements is in the order of average plant concentration. An exception to this is that the concentration of Al, Cr, Fe, and Ti in rhizome is high.

There are significant differences (P<0.05) between most of the element content (Al, B, Ba, Ca, Cu, Fe, Mg, Mn, Ti, and Zn) in the plant parts of greater celandine. The highest Al, Fe, and Ti concentration is found in the rhizome. The stem accumulates B, K, and P to the greatest extent. The leaf contains the highest amount of Ba, Ca, Cr, Cu, Mg, Na, S, and Zn, while the herb is rich in Mn. The stem contains most elements (Ba, Ca, Cr, Cu, Fe, Mg, Mn, Na, S, Ti, Zn) in the lowest concentration compared to the other parts of the plant (Table 4). The results for rhizome and herb are similar to those that we get in an earlier experiment for plants obtained from different places and years.[3]

Table 4. Element content ± standard deviation (μg g^{-1} of dry weight, n=3) in the dried plant parts of greater celandine.

Element	Rhizome	Stem	Leaf	Herb
Al	1054±13	260.7±5.0	195.8±5.6	184.1±2.7
B	22.69±0.32	71.81±0.66	29.10±4.41	19.92±0.52
Ba	21.82±0.47	8.32±0.06	22.02±0.16	16.61±0.16
Ca	15540±39	4785±12	22690±96	11723±72
Cr	2.04±0.48	0.593±0.428	2.22±0.19	2.01±0.18
Cu	17.56±0.21	12.12±0.15	19.83±0.42	13.34±0.21
Fe	814.7±14.8	77.55±1.92	234.0±2.2	216.2±1.7
K	36828±610	52346±1136	38369±504	32285±161
Li	0.864±0.360	< dl	< dl	< dl
Mg	1821±56	836.3±10.2	2132±57	1729±29
Mn	27.57±0.47	9.64±0.10	16.86±0.26	42.60±5.01
Na	297.9±4.6	218.1±4.5	522.1±10.7	298.8±2.0
P	3514±31	4911±60	3557±44	3887±515
Pb	1.86±0.43	< dl	1.56±0.79	< dl
S	1405±32	893.4±10.3	2006±7	1478±10
Ti	18.23±1.98	2.99±0.06	5.69±0.29	4.99±0.62
Zn	31.04±0.22	27.85±0.46	52.64±0.78	45.53±0.14

<dl under detection limit

The element content in the parts of greater celandine is similar to the average plant concentration except in the case of Al, Cr, Fe and Ti where the concentration of rhizome is high compared to the average plant concentration (< 200 μg g^{-1}, < 1 μg g^{-1}, < 30 μg g^{-1}, < 10 μg g^{-1}, respectively. The Cr and Fe concentrations of leaf and herb are also high, and the concentration of Ca and S in the stem is less than the average plant concentration (10-30000 μg g^{-1} and 1000-5000 μg g^{-1}, respectively).[33] The combined high concentrations of Al, Cr, Fe, and Ti in the rhizome indicate soil contamination, suggesting that the soil was not properly washed out of the sample.[34] The partly parallel change of these elements is signed by the positive correlation of Al-Fe, Al-Ti, and Fe-Ti (R=0.957, 0.971 and 0.998, P<0.05).

The coptisine content showed a tight correlation with the Cr, Cu, and Mg content in the drug (-0.989; 0.976 and -0.972; P<0.05). This is confirmed by other analytical measurements and mathematical calculations[35] that the alkaloid metabolism is regulated by some elements, for example, Mg, Cr and Zn.

Antioxidant activity of greater celandine drug

The antioxidant activity of the plant parts is shown in Table 5. The highest antioxidant value is connected to the leaf, and the lower ones are to the rhizome. There are a lot of compounds with antioxidant properties in the plant, such as vitamin C, vitamine E, flavonoids, carotenoids, polyphenols.[36] Better antioxidant value can be connected, for example, to the extracts or antioxidants with higher flavonoid (e.g., quercetin or rutin) and polyphenol content.[14] Leaves with the highest flavonoid content (Table 2) showed the highest antioxidant value, while the rhizome the least. Nevertheless, according to the correlation calculation, the antioxidant value is not correlated with the flavonoid or polyphenol content, rather a positive correlation was found with Zn content (R=0.979) at P<0.05. Zn is also of antioxidant property,[37] but the clear connection of the antioxidant value of greater celandine with its Zn content needs more data and further examinations.

Table 5. Antioxidant activity of the plant parts of dried greater celandine measured by FRAP method.

Part	FRAP value, μ mol L^{-1}
Rhizome	82.6±0.9
Stem	119.5±1.8
Leaf	426.6±2.6
Herb	325.1±2.5
Quercetin	3798±3
Rutin	5219±4

Organic agents in the greater celandine extracts

Although the rhizome contains the highest amount of alkaloids, the aerial part of the plant also has a significant amount of it, and the herb is more easily collected and does not contain higher concentrations of unwanted elements from soil. Therefore the herb of the plant was used for extraction.

The dry material and total alkaloid content of the extracts are presented in Table 6. Here it can be seen the differences that give more information for extraction as a method. The highest dry material and alkaloid content can be connected to the pressed latex, while the other extracts have similar but lower dry material content. Since the dry material content greatly depends on the amount of solvent as well, the yield of dry material may show more relevant information about the organic components of the extracts. According to this, the tea and alcoholic extract contain the highest amount of organic agents (20.18 and 20.24 %, respectively) followed by microwave extraction with water (9.88 %).

Table 6. Dry material content (%, w V^{-1}, n=3) in the greater celandine extracts, as well as the yields of dry material and alkaloids (wt. %, g 100 g^{-1}).

Method	Dry material content, g in 100 mL	The yield of dry material, wt. %	Total alkaloid content, %, g chelidonine 100 mL^{-1} extract	Yield of alkaloid, wt. %
Tea	1.211±0.025	20.18	0.060±0.015	54.4
Pressed latex	2.915±0.012	0.513	1.98±0.06	18.8
Pressed latex withwater	1.293±0.036	0.258	1.09±0.05	41.5
Alcoholic extract (96 %)	1.012±0.011	20.24	0.0164±0.02	17.7
SFE with water	1.087±0.026	1.74	0.266±0.004	23.0
SFE with propylene glycol	1.270±0.029	0.338	0.072±0.002	1.03
SFE with alcohol	1.112±0.031	0.494	0.211±0.003	3.11
Microwave extraction with water	0.988±0.013	9.88	0.049±0.001	26.5

First of all, we were interested in the total amount of effective substances in the extracts of fresh plants. The total alkaloid contents expressed in chelidonine of the different solutions are very different because of the different method applied, and the dissolution of bioactive compounds change with the seizing fluid and solvent, so it was observed the difference e.g., between the alkaloid content of aqueous and alcoholic extracts (Table 6). The tea, the alcoholic extract and the microwave extract contain the less amounts of total alkaloids. This means that the alkaloid dissolution at different rates depends on other parameters as well. If an alkaloid yield is calculated from the initial alkaloid content of fresh herb, we can see that more than half (54.4 %) of the alkaloid content of the plant is gained by tea making, 41.5 % is gained by pressed latex with water. By the use of water at pressed latex, we got a diluted extract for alkaloid, but the alkaloid yield increased from 18.8 % to 41.5 %. These results show that the pressed latex and the pressed latex with water gave extract rich in alkaloids, while the tea and the pressed latex with water gained the highest amount of total alkaloid from the plant. The SFE almost independently from the servant solvent seems not to be a good method for obtaining alkaloid rich extracts and alkaloid gaining.

The results obtained for the extraction strengthen our earlier examination on this topic that the extracts (latex, tea, microwave, SFEs) have significant effective compounds and elements, although the different parameters applied in the extractions did not give an outstanding difference in the results.[15,38] According to the correlation calculations, the dry material content has a high positive correlation with the total alkaloid content (R=0.907, P<0.05).

However, the amounts of individual components was also important for us. That's why the alkaloid combinations of extracts were observed by thin-layer chromatography and densitometry. The separation of the components was achieved by Wagner's recommended eluents.[23] The main alkaloid component contents in the extracts are summarized in Table 7. It can be seen that the main alkaloids are coptisine and chelidonine. The loss of alkaloids is high in the case of pressed latex since only trace amounts of alkaloids present in the latex (0.05-5.2 %). Nevertheless, this extract contains alkaloids in high concentrations. By supercritical fluid extraction (SFE), we didn't get homogeneous solutions, and the SFE extracts show rare alkaloid composition. They contain only a small amount of berberine, coptisine, and chelidonine (Table 7).

Table 7. Main alkaloid content (wt. %,) in the extracts measured by TLC and densitometry, and the dissolution rate of alkaloid components (%) in parenthesis.

Method	Berberine	Coptisine	Chelidonine
Tea	0.01 (69)	0.11 (80)	0.059 (2.2)
Pressed latex	0.15 (5.2)	0.45 (1.0)	0.42 (0.05)
Pressed latex (H_2O)	0.13 (38)	0.352 (11)	0.350 (0.6)
Alcoholic extract (96 %)	0.01 (83)	0.04 (35)	0.94 (43)
SFE (water)	0.071 (4.7)	0.136 (9.5)	0.12 (0.44)
SFE (propylene glycol)	0.009 (1.0)	0.063 (0.73)	0.24 (0.15)
SFE (alcohol)	0.028 (0.32)	0.555 (6.6)	0.72 (0.45)
Microwave extraction (water)	0.01 (42)	0.112 (49)	0.034 (0.77)

Alkaloid combinations of extracts represent a rare alkaloid dispertion mainly with coptisine and chelidonine, although the high berberine content of fresh latex seems to the reason for its anti-inflammatory and antiviral activity.[39] The dissolution of alkaloid components from the drug into the extracts varies to a large extent by extracts.

In some cases, a high amount of loss was observed mainly in pressed latex and SFEs, while the tea making and extraction with alcohol seem to be a good method for gaining berberine and coptisine (Table 7). The total alkaloid content in the extracts is in correlation only with their berberine content (R=0.937, P<0.05).

Inorganic elements in the greater celandine extracts

The element concentrations of the examined extracts are in Table 8. In the drug, we found a significant amount of Cr and Mn compared to the average concentration of plants. The element concentrations in the extracts are not big in view of their absolute values except for K and Ca. But anyway, it is important to know that elements are also present in the aqueous, alcoholic extracts and the pressed latexes, and these elements may contribute to the therapeutic effect of extracts.

Table 8. Element content in the greater celandine extracts ± standard deviations (μg mL^{-1}, n=3) and dissolution rate in parenthesis (%).

Element	Tea	Pressed latex	Pressed latex with water	Alcoholic extract	Microwave extract with water
Al	4.26±0.60 (39)	4.47±0.19 (0.4)	2.83±0.08 (1.1)	0.84±0.06 (9.1)	1.43±0.07 (7.7)
B	0.39±0.02 (33)	2.38±0.03 (2.1)	3.09±0.08 (10)	0.29±0.01 (29)	0.94±0.22 (47)
Ba	0.21±0.01 (21)	0.31±0.01 (0.3)	0.28±0.01 (1.2)	0.06±0.01 (7.5)	0.08±0.01 (4.7)
Ca	224±14 (32)	2028±10 (3.0)	589±15 (3.5)	139±3 (24)	488±23 (41)
Cr	0.012±0.001(9.6)	0.04±0.01 (0.4)	0.04±0.01 (1.4)	<dl	<dl
Cu	0.08±0.01 (9.8)	0.26±0.01 (0.4)	0.29±0.01 (1.5)	0.006±0.001 (0.9)	0.11±0.01 (8.1)
Fe	0.73±0.04 (5.6)	2.97±0.07 (0.2)	5.11±0.06 (1.7)	0.22±0.01 (2.0)	1.69±0.14 (7.8)
K	1518±18 (78)	7042±63 (3.8)	1928±42 (4.2)	261±4 (16)	1538±36 (47)
Mg	42.3±2.6 (41)	420±9 (4.3)	134±1 (5.4)	33.5±0.7 (39)	54±5 (31)
Mn	0.92±0.01 (36)	2.49±0.03 (1.0)	1.37±0.01 (2.3)	0.40±0.01 (19)	0.85±0.15 (20)
Na	15.6±0.4 (87)	26.3±0.4 (1.5)	8.62±0.19 (2.0)	6.2±0.4 (41)	25.8±0.6 (86)
P	179±9 (77)	612±2 (2.8)	64.3±0.9 (1.2)	40.0±0.5 (21)	38.1±1 (9.7)
S	37.8±1.2 (43)	155±1 (1.8)	57.1±0.8 (2.7)	29.6±0.6 (40)	10.3±1 (6.9)
Zn	0.10±0.01 (3.8)	2.97±0.03 (1.1)	0.94±0.01 (1.5)	0.04±0.01 (1.7)	0.08±0.01 (1.9)

<dl below detection limit

More than 70 % of the Na, K, and P content, about 40 % of the Al, Mg, and S content, more than 30 % of the B, Ba, Mn, Ca content are dissolved into the tea from the drug with the organic compounds. On an average, hardly 2-4 percent of the elements get to the pressed latex (e.g., 4.3 % Mg, 3.8 % K, 3.0 % Ca, 2.8 % P, 1.8 % S and 1.5 % Na), and it can be seen a little bit higher dissolution in the case of pressed latex with water. The dissolution rate of elements was significant in the alcoholic and microwave extracts, but their concentrations do not reach the values of tea in most cases (Table 8). Altogether the highest element dissolution was observed into the tea while the micro amounts of microelements were present (e.g., 0.04 μg g^{-1} Cr) in the locally applied pressed latex but anyhow our organism needs micro amount from some elements[40] and these small amounts depending on many factors and the circumstances can be transferred in high rate via the skin.[41]

The element content of SFEs was not analyzed because of the limited amounts of extracts, which was used for the determination of main active agents. Therefore, the element content of the residue of SFEs was determined (Table 9). Some accumulation of Al, Cu, and Na can be seen in the residues of the SFEs, which means that only a little amount of element gets into the extracts from these elements. The Ba, Ca, Cr, K, Mg, K, P, S, and Zn contents of SFE residues are lower than that of the plant, which means that the extracts contain these elements in a relatively higher amount. In our earlier examination in another kind of SFE, we obtained similar results.[15]

Some metals such as gold, copper, zinc, arsenic, silver, have antiviral activities and they effects are proved against several types of viruses, such as human immunodeficiency virus (HIV), herpes simples virus (HSV), hepatitis A, B, C, influenza.[42-44] In general the reduced transition metals (e.g. Fe, Cu, Cr, Mn) have antiviral activity and cause oxidative stress via Fenton and Fenton-like reaction.[24,45-47] It is also known for a long time that certain metals can act synergetic with other agents, bioactive molecules. Copper and gold kill microorganisms in the water in the presence of chloride.[48] The copper concentrations of the pressed extracts are of a similar order of magnitude, while the iron concentration is higher than that found to be effective (10 mM metal alone or in combination) against *Streptococcus mutans*.[45]

Table 9. Element content of the residue of SFEs made from greater celandine ± standard deviations (μg g^{-1}, n=3).

Element	SFE		
	water	propylene glycol	alcohol
Al	202.8±8.9	214.7±2.1	46.08±0.63
B	14.31±0.53	13.5±3.2	10.11±0.22
Ba	16.4±0.41	5.65±0.11	6.22±0.26
Ca	9906±152	5625±48	3456±35
Cr	1.97±0.61	0.618±0.109	2.11±0.29
Cu	14.1±1.8	17.72±0.45	17.4±5.1
Fe	218.3±9.2	115.7±0.6	77.95±2.50
K	8361±181	14883±178	12708±272
Mg	955.6±20.6	862.3±10.8	373.1±6.7
Mn	20.01±0.36	11.22±0.05	5.82±0.12
Mo	0.537±0.408	0.836±0.286	0.695±0.576
Na	266.7±11.7	344.0±19.0	282.5±5.7
P	996.0±11.7	1949±50	1093±32
S	911.3±38.6	1822±42	484.4±8.6
Ti	14.46±1.82	2.30±0.05	2.74±1.11
Zn	37.11±0.66	39.00±0.98	58.80±1.06

The copper concentration found to be effective against the HSV is 100 mg L^{-1}, [46] and this copper concentration exists in the microwave extract and a little bit higher concentration in the pressed latex extracts (Table 8). There is no information yet on the effectiveness of metals against human papilloma viruses (HPV) that cause warts although the antiviral activity seems to occur by similar mechanism in all type of viruses.[42] According to these the copper and iron concentration of pressed latex and pressed latex with water, beside their high alkaloid content, contribute certainly to their antiviral effect against warts and antibacterial activity.

Antioxidant activity of greater celandine extracts

The antioxidant activity of extracts shows that the highest values belong to the SFE prepared with alcohol (presumably due to its polyphenol content) and SFE with propylene

glycol while the pressed latex, pressed latex with water and microwave extraction with water show relatively low activity (Table 10). The antioxidant activity of extracts represents higher values mainly for SFEs compared to our earlier examinations for alcoholic extracts, where we found 90.6 and 91.4 µmol L^{-1} antioxidant activity for 20 and 40 % alcoholic extracts of greater celandine and Nadova and co-authors also published potential antioxidant activity.[27,49] So these extracts, mainly the SFEs have prominently high FRAP values.

There is no correlation of FRAP values with any other parameters. This calculation is confirmed by Khodabande and coauthors, who examined the antioxidant activity of greater celandine by the FRAP method also, and they did not find any relationship with any parameters measured.[50] This means that in the extract there are many compounds, from which one part represents antioxidant activity, while the other has prooxidant property.

According to these results it can be summarised that extracts with higher FRAP values presumable have higher content of antioxidant agents.

Table 10. Antioxidant activity of the greater celandine extracts measured by FRAP method.

Test material	FRAP value, µmol L^{-1}
Tea	299.5±2.8
Pressed latex	132.8±5.6
Pressed latex withwater	112.5±2.6
Alcoholic extract (96 %)	289.5±2.8
SFE with water	672.8±3.8
SFE with propylene glycol	2895±3.1
SFE with alcohol	3795±10
Microwave extraction with water	92.9±3.3

CONCLUSION

The supercritical fluid extraction seems to result a biomass that can be modified according to the use or stored for further processing with water, alcohol, or propylene glycol. Although there is no data for element content of SFEs, the outstanding FRAP values of aqueous and alcoholic SFEs suggest that the active ingredients, such as flavonoids with their anti-inflammatory activities and berberine or Mg, Cr, and Zn, can do much in the treatment of acne-prone skin, microtrum skin surface.

Since the greater celandine is a plant containing sensitive active ingredients, the pressed latex extracts are still the most suitable ones for gaining alkaloid rich preparations against warts. In addition to the high alkaloid content, these extracts also contain transition metals (e.g., Cu, Fe) in concentrations that are effective against viruses. In summarising the metal element content of the pressed extracts presumably contributes to the beneficial antiviral effect of greater celandine. The other methods made with water (tea, microwave extract) also give the extracts with relative high alkaloid and transition metal content.

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