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Capillary electrophoresis with led-induced native fluorescence detection for determination of isoquinoline alkaloids and their cytotoxicity in extracts of *Chelidonium majus* L

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ABSTRACT

In this study, we introduced a simple and sensitive method of capillary electrophoresis with ultraviolet light-emitting diode-induced native fluorescence (UV-LEDIF) detection for the determination of isoquinoline alkaloids in extracts of *Chelidonium majus* L. Samples were extracted with acidic methanol and the extracts were directly analysed by CE. Simultaneous determination of protopine, chelidonine, coptisine, sanguinarine, allocryptopine, chelerythrine and stylopine was performed in 20 mM phosphate buffer (pH 3.1). The baseline separation of these alkaloids was finished within 20 min. As these alkaloids have native fluorescence, they were directly detected using the commercially available UV light emitting diode without troublesome fluorescent derivatisation. Satisfactory LOD values were obtained for the studied compounds considering their appearance in natural extracts. Lower limits of detection were 0.05 μg/mL for protopine, 0.06 μg/mL for stylopine and allocryptopine, 0.07 μg/mL for chelidonine, 0.22 μg/mL for sanguinarine, 1.7 μg/mL for chelerythrine and 5.5 μg/mL for coptisine. The developed method was successfully applied to determine the contents of seven alkaloids in the aerial parts of *Chelidonium majus* L, which varied from 0.025 to 0.763% (w/w). Also, to demonstrate the potential of the proposed CE method, an estimation of the cytotoxic properties of selected Celandine alkaloids in a natural extract was carried out.

1. Introduction

Alkaloids are important class of compounds that have pharmacological effects on various tissues and organs of humans and other animal species. In the past 10 years, there has been an increasing interest in the isolation and determination of alkaloids in plant materials, in pharmaceutical products, and in other samples. Currently much work is being carried out to discover new alkaloid molecules for different applications [1,2], such as new antiviral and anti-tumour treatments. Therefore, the separation and analysis of alkaloids are of great importance.

The greater celandine (*Chelidonium majus L.*), an herb of the poppy family and native to Europe and Asia, is a rich source of biologically active substances used for the treatment of various diseases. It has been demonstrated that both alkaloid extracts and purified alkaloids from that plant exhibit distinct anti-inflammatory, anti-microbial, and anti-tumour activities [3–5]. The most effective alkaloid components of the plant are protopine, chelidonine, coptisine, sanguinarine, allocryptopine, chelerythrine, etc. (Fig. 1). Determination of these individual alkaloids is important in developing and utilising resources of greater celandine, as well as in better understanding the mechanism of the biological action of specific alkaloids.

A variety of analytical techniques which enable separation and quantification of alkaloids are currently available. Application of separation methods such as high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE) for this purpose is a routine procedure with large number of modifications [6–9]. Kursinszki et al. [6] described an HPLC method with a reversed-phase column for determination of five alkaloids in *C. majus* extracts. However, ionic interactions of the basic alkaloids with three silanol groups on the silica surface can contribute to peak tailing during separation on a C-18 phase. More time-consuming and/or expensive procedures exploring specific HPLC columns with reduced silanol activity and gradient elution [7] or ion-pairing [4] HPLC have also been proposed. But complicated operation, including the necessity of sample pretreatment with solid-phase extraction in the case of complex natural extracts, consumption of a large amount of organic solvents and long analysis time make HPLC methods rather inconvenient.

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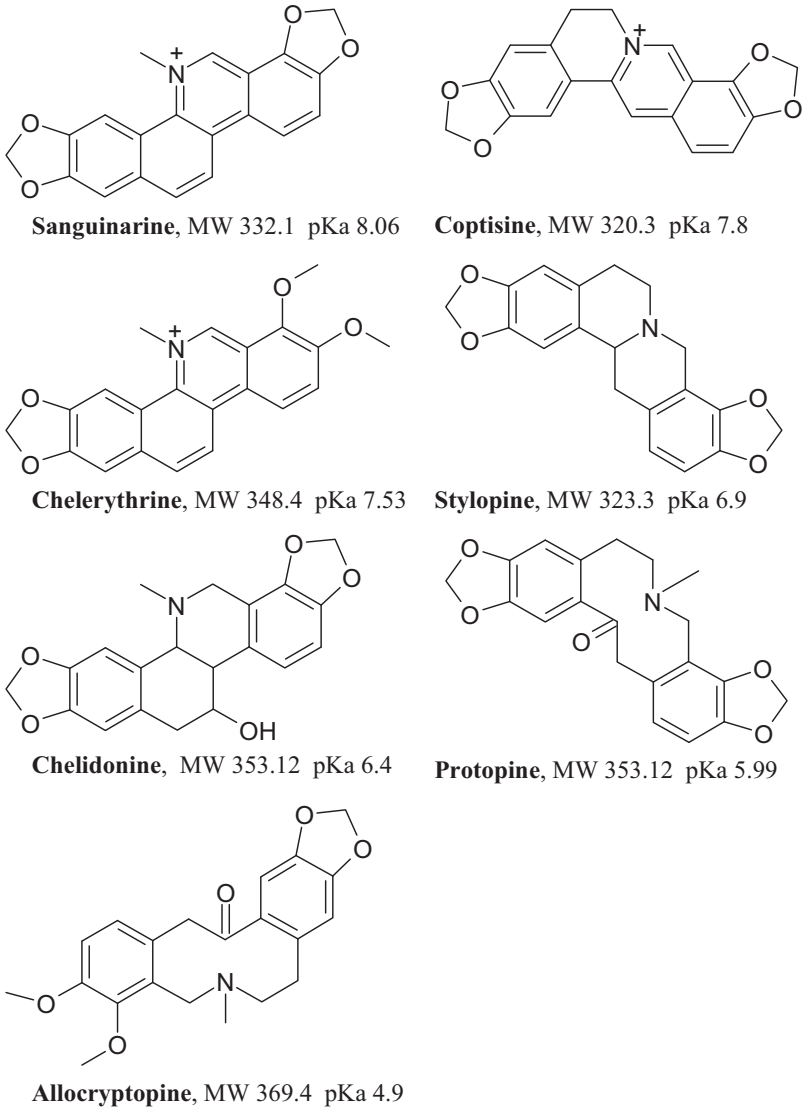

Fig. 1. The chemical formulae, molecular masses and pKa of seven alkaloids of greater celandine.

After its introduction, capillary electrophoresis has been proven to be one of the most powerful tools for the analysis of small molecules such as pharmaceuticals and natural products. It has many advantages such as high separation efficiency, short run time, instrumentation simplicity, minimum operation cost and compatibility with small sample volumes. UV-absorbance is the major detection approach in CE analysis of alkaloids [10–12]. Absorbance detection can be applied for different types of alkaloids and does not require derivatisation of the analytes prior to injection. The preferred spectral band for absorbance detection of alkaloids is 280 nm. It is associated with the absorption of aromatics usually presented in alkaloid molecules. However, UV detection at 280 nm suffers from high background and interference from other species.

Alternatively to UV detection, laser-induced fluorescence (LIF), one of the most sensitive methods, is widely used for CE analysis [13]. Generally, to reach maximum sensitivity, the fluorescence wavelength of analyte is required to match that of the laser. Most drugs lack a suitable laser to excite their native fluorescence. Therefore, has become a common trend to chemically derivatise drugs with a fluorescent label to make them detectable. But fluorescence derivatisation is usually a complicated and time-consuming process and many drugs, including most alkaloids, can still not be derivatised well due to the lack of a reaction group or proper fluorescent label. Especially for alkaloid analysis in plants, derivatisation is not a wise choice due to the complicated matrix of ingredients [14]. Also, a number of other limitations such as high cost, limited lifetime and high power consumption greatly limit the application of LIF detection.

A simple and inexpensive light source with promising applications for fluorescence measurements is the light-emitting diode (LED) [15,16]. The combination of extremely high stability, long lifetime, small size, low cost and commercial availability at wavelengths ranging from deep-UV to near-IR regions make LEDs an attractive light source. Indeed, different LEDs have been used recently for the analysis of proteins, peptides and DNA fragments [17–21]. Determination of different amino acids and riboflavin by capillary electrophoresis has been carried out using single- and multi-wavelength array LED fluorescence detection [22–24]. A UV and blue LEDs have been used as the excitation sources for the analysis of biogenic amines and PAHs [25,26]. Tobacco alkaloids have been determined in tobacco extracts using a 405 nm LED and electrochemiluminescence detection [27]. CE studies published so far mostly use blue or UV LEDs as excitation sources and the fluorescent labelling of the analytes. Deep-UV LED [18] allows detecting native fluorescence of many types of compounds, such as proteins, peptides and alkaloids without the need for derivatisation. In the

present study, we demonstrate an application of capillary electrophoresis with deep-UV LED detection operating at 280 nm for the analysis of isoquinoline alkaloids in extracts of the *Chelidonium majus* plant. In addition, in order to demonstrate the potential of the developed CE method, the cytotoxic activity of selected alkaloids from natural celandine extract was investigated.

2. Experimental

2.1. Chemicals

Standards of the alkaloids chelidonine, sanguinarine, allocryptopine and chelerythrine were obtained from Sigma–Aldrich (USA); stylopine, protopine and coptisine were purchased from LGC Standards (UK). Sodium phosphate, phosphoric and hydrochloric acids and methanol were also obtained from Sigma–Aldrich (USA). Water was purified with Millipore (USA) Milli-Q equipment.

2.2. Plant material

The aerial parts of *Chelidonium majus* L. were collected from fields and forests near Tallinn in May 2010. Plant material was then dried and powdered.

2.3. Sample preparation

Standard solutions of chelidonine, sanguinarine, allocryptopine, chelerythrine, stylopine, protopine and coptisine were prepared in methanol containing 0.05 M hydrochloric acid.

0.5 g of *Chelidonium* aerial parts were extracted six times with 40 mL of methanol containing 0.05 M hydrochloric acid by sonication at $27 \pm 2^\circ$ C (Sonorex, Bandelin, Germany) for $6\times$ 10 min. The extracts were separated from the plant powder by centrifugation at 6000 rpm for 15 min. After the last extraction, there was no residue after evaporation of the solvent and no peaks were detected by CE in the extract. The six methanolic fractions were combined and evaporated to dryness under reduced pressure (Laborota 4000, Heidolph, Germany) below 60 °C. Samples for CE analysis were prepared by dissolving a part of this extract in methanol containing 0.05 M hydrochloric acid.

2.4. LED-fluorescence detector and CE apparatus

The LED-fluorescence detector for capillary electrophoresis was designed and constructed by Laser Diagnostic Instruments AS, Estonia. The UV-LED (Roithner Lasertechnik, Austria) was used as the fluorescence excitation source ($\lambda = 280$ nm). The LED radiation had a power output of 0.5 mW and spectrum half width of 15 nm. The temperature of the LED was adjusted using an external cooling module with a programmable temperature controller. This controller keeps a constant temperature (accuracy ± 0.5 °C) when the ambient temperature fluctuates. The cooling module was used for LED operation stabilization. LED optical oscillations at different wavelengths were separated by an interference filter (280 nm, band width 10 nm). The excitation radiation was focused onto the tilted capillary by a lens (EFDS-280, LDI). The fluorescence signal was collected at a 90° angle to excitation by a second lens (EFDS-280, LDI) onto a photon counting PMT (H7467, Hamamatsu, Japan) (integration time up to 10,000 ms). The detector is supplied with MCF Analyzer software (LDI) which is capable to store, print and display ongoing sample measurements. Two interference filters (307 and 326 nm, Andover Corporation, USA) with band width 10 nm and wideband filter 341–600 nm (BrightLine® long-pass filter FF01-341/LP, Semrok, USA) were mounted before the photon counting PMT and used to block reflected UV-radiation and select the required spectral region. In the present study, a wideband filter 341–600 nm was used to register the fluorescence signal.

The CE apparatus was constructed in-house. A polyimide-coated fused silica capillary, i.d. 75 μm and o.d. 360 μm (Agilent Technologies, USA) was used for the analyses. The total capillary length was 65 cm with the detection zone situated 15 cm from the capillary end.

The fluorescence spectra were recorded using a Hitachi module F-7000 fluorescence spectrophotometer. The excitation wavelength was fixed at 280 nm and the emission wavelength scanned from 200 to 600 nm.

A Q-Star Elite mass spectrometer (Applied Biosystems, Germany) equipped with an ESI source was used for Q-TOF experiments. Mass spectra were obtained in a full scan mode 150–1000 amu). The instrument was operated in a positive ion mode under the following conditions: needle voltage, 5.5 kV; ion source gas (N2), 27; curtain gas (N2), 20. To obtain the full positive spectra, the sample was diluted 100 times with a mixture of 50% methanol + 0.1% acetic acid + deionised water and introduced into the mass spectrometer by a syringe.

2.5. CE analysis

Prior to injection, the capillary was rinsed sequentially with 0.1 M NaOH, water, and running buffer for 2 min each. Filtered samples were injected into the capillary by hydrodynamic flow at a height differential of 10 cm for 10 s. Separations were performed at 16 kV using a background electrolyte solution containing 20 mM sodium phosphate (pH 3.1) and quantification of alkaloids was achieved by measuring the peak areas.

2.6. Cell culture

The murine fibroblast NIH/3T3cell line, obtained from American Type Culture Collection (ATCC) was propagated in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% bovine calf serum and 5% penicillin/streptomycin and incubated at 37 °C in a humidified 5% CO_2 and 95% air atmosphere.

2.7. Cytotoxicity assay procedure

The cells were plated at a density of 5×10^5 cells/cm^2 into a 24-well plate and incubated overnight. After 24 h of incubation, 250 mL of new media or new media with alkaloid extract (the total concentration of alkaloids was 11 μg/mL) was added, and the plate was incubated for a further 45 min. As a control for capillary electrophoresis analysis, the medium with alkaloid extract without cells was incubated under the same conditions. The media with alkaloid extract and controls (three parallels for each type) were collected, precipitated with 50 μL of trichloroacetic acid and analysed by the developed CE-LEDIF method.

The cytotoxic activity of the *Chelidonium* extract was also determined by the MTT colourimetric assay [28], which is based on the mitohondrial reduction of tetrazolium salt by living cells. The viable cell number is proportional to the production of the formazan salts. For this assay, the cells were washed with FBS-free medium and MTT in an amount equal to 10% of the culture medium volume was added. After incubation for 3 h at 37 °C, the medium was discarded, the crystals of formazan blue were dissolved in 300 μL of acidified isopropanol and the optical density was measured spectrophotometrically at 570 nm (Tecan, Magellan Microplate reader, series 750). Optical density of the control untreated cells was taken as 100% viability.

3. Results and discussion

3.1. Fluorescence emission spectra of isoquinoline alkaloids

The fluorescence emission spectra of chelidonine, protopine, allocryptopine, stylopine, sanguinarine, chelerythrine and coptisine were first investigated to estimate the feasibility of this method. It was found that all seven alkaloids emitted fluorescence in the range of 341–600 nm when being excited at 280 nm. Therefore, it was possible to use a 280 nm light-emitting diode with a wideband filter (341–600 nm) to detect them in CE. Chelidonine, protopine, allocryptopine and stylopine exhibited bands around 360 nm, sanguinarine, chelerythrine and coptisine exhibited bands around 570 nm when being excited at 280 nm. The $\lambda_{ex}/\lambda_{em}$ of chelidonine, protopine, allocryptopine and stylopine perfectly matched that of the detector and maximum sensitivity could be reached. The relative fluorescence intensity of the other alkaloids was also sufficiently high. So, in the present study, the native fluorescence of alkaloids was directly detected in CE without the need for fluorescent derivatisation.

Also, a pH dependence of fluorescence intensity of all alkaloids studied was investigated. For chelerythrine, chelidonine and sanguinarine an increased pH of the buffer is beneficial as the fluorescence intensity increases compared to low pH. At pH 3.1, used in this work, coptisine, allocryptopine, protopine and stylopine provide maximum fluorescence signal. In present study, the best separation of alkaloids was achieved at buffer pH 3.1, with considerable loss of separation efficiency at higher pH. Therefore, inspite of the fact that fluorescence intensity is partially lost for chelerythrine, chelidonine and sanguinarine at pH 3.1, these alkaloids still provide enough fluorescence to be efficiently separated, detected and quantified.

3.2. Separation of alkaloids by CE

The isoquinoline alkaloids studied in the present work represent strongly basic molecules with a heterocyclic-bound nitrogen and exhibit pKa values above 5 (see Fig. 1). Thus, in acidic electrolytes, the alkaloids are protonated at the nitrogen atom and migrate as cations towards the cathode. In most published papers so far, the CE separation of alkaloids has been conducted in acidic phosphate [10], ammonium [29,30] or sodium acetate [11] buffer with the addition of acetonitrile or methanol in different ratios. On account of its good buffering capacity at low pH values, phosphate was chosen as a promising counter-ion for the alkaloids. We prepared aqueous electrolytes containing 20, 40, 60 or 100 mM sodium phosphate at pH values ranging from 2.0 to 3.5 and between 5.0 and 7.0. The best selectivity and resolution was obtained using 20 mM phosphate buffer, pH 3.1 (Fig. 2). Under these conditions, the electroosmotic flow enhanced the migration velocity of the alkaloids because this bulk flow was also directed towards the cathode.

The migration order can be derived from the molecular mass and the pKa value which determines the net charge of the molecules. According to their basic properties and their molecular masses, the isoquinoline alkaloids can be divided into three groups with decreasing electrophoretic mobility: (I) alkaloids with medium molecular mass but strongly basic properties, e.g. sanguinarine, coptisine, chelerythrine; (II) alkaloids with medium molecular mass and medium basic character, e.g. stylopine, chelidonine, protopine; and (III) a compound characterised by a relatively high molecular mass and feeble basic properties, allocryptopine.

Surprisingly, the migration velocity of sanguinarine was higher than the migration velocity of coptisine (Fig. 2). Assuming that the both molecules were fully protonated at the present pH value of the applied buffer, the higher molecular mass of the sanguinarine should have led to a lower electrophoretic mobility than for coptisine. The higher migration velocity of sanguinarine was obviously due to the presence of an additional methyl group at the nitrogen, which prevented the formation of intermolecular forces between the nitrogen atom and the solvent molecules of the phosphate buffer medium. Thus, the resulting degree of solvation was lower and the mass-to charge ratio decreased. The separation of chelidonine and protopine was impressive because they are molecules with the same mass and charge at the applied buffer pH 3.1. Apparently, slightly different basic properties and the presence of the electronegative oxygen atom, which shifts electron density from the nitrogen in protopine molecule, caused the difference in migration velocities of these alkaloids.

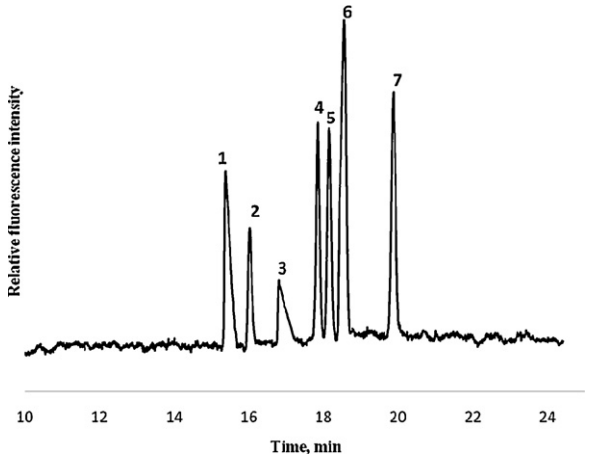

Fig. 2. CE-LED separation of alkaloid standards. Buffer: 20 mM phosphate, pH 3.1. Separation capillary: 75 μm i.d. uncoated fused-silica, 50 cm in length (40 cm effective length). Applied voltage 16 kV. LED excitation wavelength 280 nm, fluorescence recorded with wideband filter (341–600 nm). Peaks: (1) sanguinarine (40 μg/mL), (2) coptisine (250 μg/mL), (3) chelerythrine (100 μg/mL), (4) stylopine (10 μg/mL), (5) chelidonine (10 μg/mL), (6) protopine (15 μg/mL), (7) allocryptopine (10 μg/mL).

3.3. Validation of the method

The CE method with LED-fluorescence detection was validated in terms of the linear dynamic range, limit of detection (LOD), linearity, intra-day and inter-day precision.

The local environment has a profound effect on the emission spectral properties of fluorophores. Therefore, in order to avoid the complication of matching the matrix of the standards to that of the plant extract, calibration was performed by the method of standard additions. For this, celandine extract samples were spiked at different concentration levels. Calibration curves were obtained by considering the peak areas as a function of the analyte standard concentration. Each concentration level was injected in triplicate. A good relationship was obtained between the concentration injected and the uncorrected peak area for each component within the investigated range. The limit of detection (LOD) was considered as the value of three standard deviations of the extract measured five times. Good LOD values were obtained for the studied compounds considering their possible concentration in natural extracts. The LODs for sanguinarine, stylopine, chelidonine, protopine and allocryptopine were 0.22, 0.06, 0.07, 0.05 and 0.06 μg/mL, respectively. They are an order of magnitude lower than LODs of tobacco alkaloids, determined by CE with violet LED fluorescence detection [27]. The relatively high LODs which appeared for coptisine and chelerythrine were due to the chosen emission filter bandwidth which could be changed if a lower limit of detection is needed. All the results are summarised in Table 1.

Table 1
Statistics and performance characteristics of the proposed method.

Compound	Regression equation	Correlation coefficient, r	Linear dynamic range (μg/mL)[a]	LOD (μg/mL)
Sanguinarine	$y = 13{,}801x + 134{,}381$	0.992	9.7–29.7	0.22
Coptisine	$y = 538x + 55{,}845$	0.996	103.7–253.7	5.5
Chelerythrine	$y = 1754x + 9539$	0.998	5.4–25.4	1.7
Stylopine	$y = 46{,}765x + 224{,}858$	0.995	4.8–24.8	0.06
Chelidonine	$y = 37{,}626x + 164{,}079$	0.993	4.4–24.4	0.07
Protopine	$y = 48{,}451x + 320{,}507$	0.996	6.6–26.6	0.05
Allocryptopine	$y = 42{,}538x + 207{,}279$	0.999	4.9–24.9	0.06

[a] Linear dynamic range is measured between the alkaloid concentrations found in extract up to the maximum concentrations used in standard addition calibration.

The reproducibility of the method was evaluated by injecting the standard solution five times in one day and recording peak areas. To determine inter-day variation (repeatability), the standard solution was analysed twice a day on three consecutive days. The determined relative standard deviations (RSD%) were in the range of 0.5–2.8% and 0.67–3.5%, respectively.

3.4. Analysis of plant extracts

The proposed method was applied for the determination of sanguinarine, coptisine, chelerythrine, stylopine, chelidonine, protopine and allocryptopine in *Chelidonium majus* extract. For this, the filtered methanolic extract was directly injected onto the CE column without any sample pretreatment or clean-up. Due to the selectivity of native fluorescence detection, a very selective alkaloid pherogram was obtained (Fig. 3). The peak identification was assured by the standard addition method. The peak (x) owing the most intensive signal on the pherogram could not be identified by spiking due to the lack of a proper standard. However, according to the published data [7], we speculate that the unidentified compound could be a chelirubine (Mw 362.3). Under acidic conditions, this alkaloid possesses a positive charge on the iminium ion (Fig. 1) and its mass-to-charge ratio is in accordance with the migration order of the peak after protopine (Mw 353.4) and before allocryptopine (Mw 369.4). This assumption was partly confirmed by ESI-MS analysis of the extract providing the presence of molecular ion with the m/z 362 in the mass spectrum.

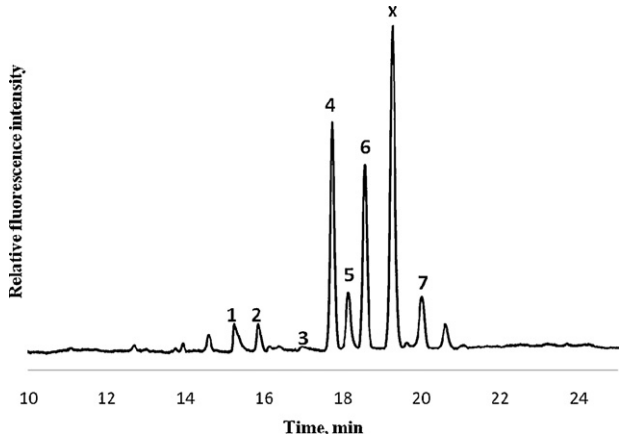

Fig. 3. Representative CE-LED pherograms of alkaloids in an extract from the aerial parts of *Chelidonium majus* L. Peaks: (1) sanguinarine, (2) coptisine, (3) chelerythrine, (4) stylopine, (5) chelidonine, (6) protopine, (7) allocryptopine, (x) unknown component. Conditions: 20 mM phosphate buffer, pH 3.1. Separation capillary: 75 μm i.d. uncoated fused-silica, 50 cm in length (40 cm effective length). Applied voltage 16 kV. LED excitation wavelength 280 nm, fluorescence recorded with wideband filter (341–600 nm).

The content of alkaloids in the extract was calculated from the calibration curves and expressed as the mass percentage of the dry aerial parts of the plant. The obtained contents of sanguinarine, chelidonine and protopine in the *Chelidonium* aerial parts naturally growing in Europe were in good agreement with the published data [6]. The content of main alkaloid coptisine obtained by CE method was two times higher comparing to HPLC study, which can be explained by the harvesting time of the plant. The alkaloid content in the aerial parts of Chelidonium majus plant can change sufficiently from the flowering period in spring to the friut-bearing time in autumn. Autors collected the plant during the flowering period, when the content of main alkaloid coptisine is supposed to be maximal. The results are presented in Table 2.

3.5. Cytotoxicity test

The biological and anti-proliferative activities and pharmacological applications of celandine alkaloids have been extensively investigated in clinical studies [31,32]. Traditional methods for the evaluation of cytotoxic activity are based on the estimation of the ratio of live and dead cells using light microscopy or spectrophotometry. First, the cytotoxic potential of the whole extract on NIH 3T3 cells was determined by a colourimetric MTT assay. For this, 5 μL of extract (70 mg/mL) was added to 250 μL of cell medium and incubated for 45 min at 37° C. Whole extract treatment increased the percentage of dead cells by about 60% after 45 min of incubation in culture. This traditional method allows for investigating the total effect of the whole extract on cell viability, or cells can be tested against a particular alkaloid alone.

However, the estimation of the specific capacity of each alkaloid in the natural extract to inhibit cell growth is very important in understanding the mechanisms of their cooperative biological action. Alkaloid cytotoxic properties are related to their ability to interact with important enzymes and other proteins and inhibiting their activity, or to intercalate into DNA by inserting their planar molecular structures between the adjacent base pairs, which leads to DNA damage. As a result of these chemical interactions, the concentration of free alkaloid molecules in the cell medium during incubation decreases proportionally to the cytotoxic capacity of

Table 2
Alkaloid content ($n = 3$) in the aerial parts of *Chelidonium majus* L. determined by different methods and expressed as the mass percentage of the dry plant.

Compound	Alkaloid content in *C. majus* (%, w/w)	
	CE-LEDIF	HPLC-UV [6]
Sanguinarine	0.055 ± 0.002	0.041 ± 0.002
Coptisine	0.763 ± 0.017	0.361 ± 0.008
Chelerythrine	0.040 ± 0.001	–
Stylopine	0.038 ± 0.001	–
Chelidonine	0.025 ± 0.002	0.027 ± 0.001
Protopine	0.049 ± 0.001	0.043 ± 0.002
Allocryptopine	0.028 ± 0.002	–

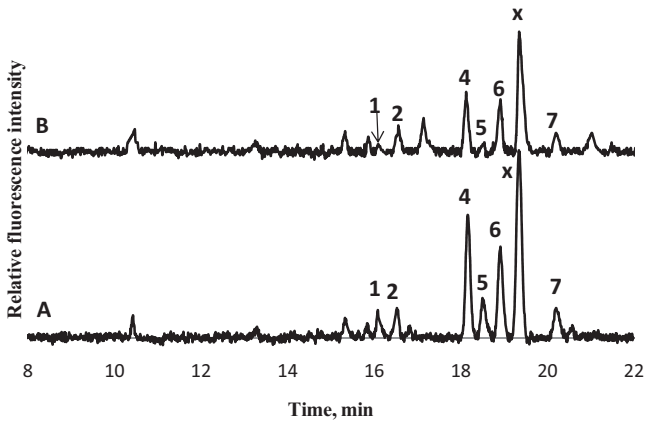

Fig. 4. Electropherograms of control medium with alkaloid extract without cells (A) and containing cells (B) after 45 min of incubation. For experimental conditions, see Fig. 3. Peaks: (1) sanguinarine, (2) coptisine, (4) stylopine, (5) chelidonine, (6) protopine, (7) allocryptopine, (*x*) unknown compound.

alkaloids. CE allows for investigating the effect of each alkaloid in the extract on cell viability by performing the analysis of extract composition before and after applying it to cultured cells. Thus, we used the proposed CE method to evaluate the cytotoxic activity of selected alkaloids by monitoring of alkaloid concentration decrease after treatment of murine fibroblast NIH/3T3 cells with *C. majus* extract.

Representative electropherograms of the medium containing alkaloid extract without cells (control) and following treatment of cells after 45 min of incubation is shown in Fig. 4. Most of the alkaloid peak intensities decreased in comparison with the extract electropherogram before the experiment, meaning that the cells indeed interacted with the toxic alkaloids.

The decreased amount of alkaloids is presented in Fig. 5. The content of each alkaloid in the control sample was taken as 100%. This figure demonstrates the differential decrease of alkaloid peak areas which indicates their different ability to interact with cells. The most effective concentration decrease was observed for sanguinarine (~70%) which was in good agreement with reported data concerning its cytotoxicity [25]. Sanguinarine contains a reactive imine group and exhibits a significant inhibitory effect on mitochondrial respiration. Chelidonine, known as an inhibitor of tubulin polymerisation [33], also showed significant interaction activity (~65% decrease). Coptisine and *x* (*chelirubine*) showed the lowest concentration decrease, around 30%, compared to the control sample. However, the coptisine content in the extract was an order of magnitude higher, compared to the other alkaloids,

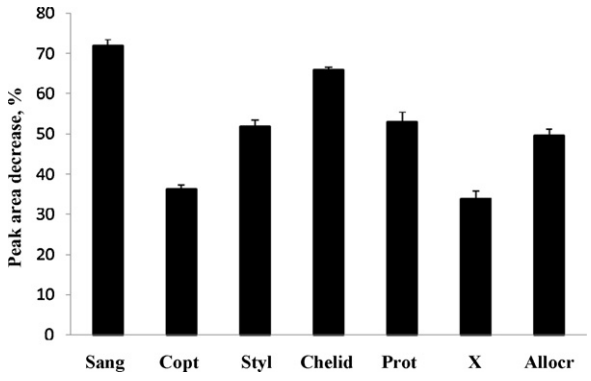

Fig. 5. The decrease of alkaloid content in cell medium expressed as percent of peak area during 45 min of incubation with murine fibroblast NIH/3T3 cells.

which indicated a significant consumption of this component by the cells. This differential cytotoxic effect of celandine alkaloids is of great interest in biomedical studies of their anti-tumour properties. In our further investigations, we are planning to demonstrate the applicability of capillary electrophoresis for this purpose.

4. Conclusions

A rapid and simple method of capillary electrophoresis with ultraviolet light-emitting diode induced native fluorescence (UV-LEDIF) detection was presented to determine the protopine, chelidonine, coptisine, sanguinarine, allocryptopine, chelerythrine and stylopine in extracts of *Chelidonium majus* L. The proposed separation protocol in combination with highly selective and sensitive LED-fluorescence detection resulted in baseline separation of seven alkaloids and enabled us to quantify them in a complex matrix without sample clean-up, which has not been possible so far (e.g. using reversed phase HPLC, sample SPE pretreatment was needed). The obtained values for the alkaloid content in the plant extract appeared to be consistent with previously reported data.

Simplified operating conditions and excellent selectivity made the method very efficient for the analysis of these alkaloids in biological samples. The well-known cytotoxic properties of isoquinoline alkaloids were demonstrated here using the developed CE method. The apparent decrease in alkaloid concentration during incubation of *C. majus* extract with murine fibroblast cells indicated the ability of these compounds to interact with cells and inhibit cell grow, which was also confirmed by a traditional MTT assay. An application of this method for the investigation of celandine alkaloid cytotoxicity on cancer cells would be an interesting direction for further development.

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