Ph.D. Thesis

Novel data contributing to our knowledge of the greater celandine

(*Chelidonium majus* L.)

Prepared by: Ágnes Sárközi
Supervised by: Dr. Ágnes Kéry, Ph.D.
Semmelweis University, Department of Pharmacognosy

**SUMMARY**

The greater celandine is a medicinal plant well-known for the spasmylytic, anti-inflammatory, antimicrobial and antitumor effects of its isoquinoline alkaloids. Although this medicinal herb is a subject of recent investigations its successful therapeutic applications has not been corroborated so far by scientific results. Re-evaluation of the aerial parts of the plant has become especially timely with its adoption as an official drug in the VIIIth Hungarian Pharmacopoeia. We wished to offer new data to the plant database, in order to ensure the pharmaceutical and therapeutic quality of *Chelidonium* drugs and preparations.

The application of *Chelidonium* drugs obtained by collection always involves higher risks than those of cultivated drugs, therefore herbal samples from our own collection have been qualified according to pharmacopoeia.

Determination of the total alkaloid content by spectrofotometry has been modified so as to allow the measurement of tertiary and quaternary alkaloids as well. The main steps and results of determination have been confirmed by HPLC assessment.

For the investigation of main alkaloids – usually carried out by HPLC method, a highly accurate but rather laboursome procedure– taking the advantage of the fluorescence property of quaternary alkaloids we developed a simple, low-cost TLC-densitometry method. This is applicable for routine determination of a large amount of samples (sensitivity: RSD = 0.67 – 1.24% and accuracy: RSD = 3,3 – 4,8%) (1).

For a closer knowledge of alkaloid ratios, important from the aspect of total alkaloid content and therapeutic effect we supplied data obtained by chromatographic methods. Measurement of the total alkaloid content of the plant parts gave the following results: generative organs (1,54%), root (1,43%), leaf (0,71%), stem (0,68%). The main alkaloid of the aerial part was determined as coptisine. In the root part, chelidonine can be found in highest amount, but the concentration of sanguinarine, coptisine, chelerythrine and berberine is also considerable. Highest total alkaloid content was measured in samples collected during the rest period, therefore this time is most appropriate for the collection of both the herb and the root.

In order to contribute to the phytotechnology of *Chelidonium* preparations, we measured the results of alkaloid dissolution from traditional extracts of the herb.

The mineral element composition of *Chelidonium* drugs and their traditionally applied extracts were analysed by ICP-OES for Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Ti, V and Zn content (2).

Our biological studies performed by the BioArena method confirmed the antibacterial activity of *Chelidonium* alkaloids and also support the assumption that the reaction mechanism is realized by the formation of formaldehyde due to demethylation (3).

1. INTRODUCTION AND AIM OF THE STUDY

Therapeutic application of herbal products yielded several significant results, which contributed to the development of modern medical science, and the application of herbal products in their original form is nowadays still increasing. In this process a repeated recognition of the advantages of traditional medicinal herbs, which have been included in the national pharmacopoeias for a long time and on the basis of long years' experiences proved to be effective both in its original and processed form as components of medicinal products, played a dominant role. The greater celandine (*Chelidonium majus* L.) is such a medicinal herb having been applied from time immemorial and as proved by a large number of publications in the literature, it serves as the subject of recent investigations as well.

In the last decade the application of medicinal herbs has been increased all over the world and the reason for this progress – besides many others – is the ever-growing confidence in herbal products against artificial medicines and the approach wherein the preference for “safety” has also joined to the preference for “natural”.

In order that the database compiled to satisfy the increasing demand for information does not only consist of the knowledge of traditions and experiences arising from the traditional use of medicinal herbs, there is a need for scientific results-supported phytochemical and pharmacological studies of medicinal herbs, and even more of herbal medicine products.

The *Chelidonium majus* L. is included in several pharmacopeias and monograph collections and in Hungary; the aerial part of the plant has been first declared an official drug in the Hungarian Pharmacopoeia (Ph. Hg. VIII.) that became effective on 1 August 2006. In the meantime, the demand for professional information about the greater celandine, its drugs (Chelidonii herba and radix) and the approved products derived from this herb is continuously increasing.

While collecting the botanical, phytochemical and pharmacological data on the greater celandine, we became convinced of the need for summarizing the available abundant information and the expediency of conducting further phytochemical and biological researches.
Our aim was to develop quick and simple methods for the phytochemical and biological investigation of the drugs and products derived from the Europe-wide used *Chelidonium majus* L. and to contribute to a more safe phytotherapeutic application and the further development of medicines.

The investigation of *Chelidonium* alkaloids was the main subject of our researches since these isoquinoline alkaloids of the greater celandine are responsible for the spasmolytic, anti-inflammatory, antimicrobial and anti-tumor effects. We also attached importance to the adaptation, critical evaluation, comparison and the required amendment of the appropriate methods for the quantitative measurement of certain alkaloid compounds, besides the measurement of the total alkaloid content that is officially described in the pharmacopoeias (DAB 10, Ph.Eur.5., Ph.Hg.VIII.). For the investigation of the main alkaloids – besides the precise but laboursome and time-consuming HPLC assessment – we deemed it proper to develop a simple, low-cost TLC-densitometric method that is applicable for routine determination of a large amount of samples. Taking the advantage of the fluorescence property of alkaloids we expected to increase the accuracy of the densitometry method in order to find an alternative method of the HPLC.

A further aim of our investigations was to study the dissolution of alkaloids in aqueous and alcoholic medicines, which have a great importance and are widely used application forms of medicinal herbs. Due to the complex chemical structure it was required to apply a highly effective and sensitive analytical method. The method was optimized in order to be able to assess the different extracts besides the analysis of the drug.

During our biological investigations we found that the micro- and macroelements of the plant have an influence on the effect. Since we could not find an adequately detailed and accurate minerals determination method already published in connection with the *Chelidonium* drugs, our aim was also to determine a modern, inductively captured plasma emission spectrophotometric method for assessing mineral contents of aqueous and alcohol extracts prepared according to the requirements of traditional medicine manufacturing.

The exact collection time of *Chelidonium* drugs has not been determined in the valid pharmacopoeias and the published recommendations are inconsistent and not confirmed by adequate results. Therefore we attached importance to investigate the change of total
alkaloid content during the vegetative period and the ratio of alkaloids relevant from the aspect of the therapeutic effects; and to provide data that have been defined by modern chromatographic method for the determination of the exact collection time. The antiviral, antibacterial and antifungal effect of alkaloids and extracts obtained from *Chelidonium* are widely known in the literature. Isoquinoline alkaloids of the greater celandine are N and O-methylated and these groups can be easily detached from them, so as these molecules are potential sources of formaldehyde. For the above mentioned reasons it was deemed to be promising both to accomplish an antibiotic effect analysis by BioArena system of *Chelidonium* alkaloids and to investigate our assumption that the alkaloids exert their effect through formaldehyde release after demethylation.

2. MATERIALS AND METHODS

2.1. Plant material

Our investigations were performed on self collected *Chelidonium majus* L. (Papaveraceae) samples. The development of the analytical methods (TLC-densitometry, spectroscopy, HPLC), procedures on the different parts of the plant material (leaf, stem, generative part [inflorescence and fruit], root), and the determination of the alkaloid and mineral element content of the extracts obtained on the basis of the traditional application of medicinal products (decoction, infusion, and 40, 70, 90 V/V% ethanol tinctures) were performed utilising plant material which was collected in 2004 in Budapest (Hűvösvölgy) (sample A) and in Nagymaros (sample B). Investigations in the changes of the alkaloid content during the growing years were performed on plant material was collected in the territory of Budapest (Hűvösvölgy) and Budakeszi 10 times per 20 days between the beginning of April and October 2005. Plant material was identified as *Chelidonium majus* in the Department of Pharmacognosy, Semmelweis University where a herbarium specimen has also been deposited.
2.2. Extraction methods and sample preparation

2.2.1. APPLICATION OF TRADITIONAL MEDICINAL PRODUCTS

Owing to the mostly available data about the traditional application of medicinal products Chelidonium herba and different tinctures were used to make aqueous and alcoholic extracts from plant drugs. For preparation of extractions the drug end solvents were used in the ratio of 1:40. We applied two different kinds of aqueous extracts (decoction, infusion) and three alcoholic tinctures (40, 70, 90 v/v%).

2.2.2. PRODUCTION OF LYOPHILIZED SAMPLES

The aqueous extracts (decoction, infusion) and the tinctures (40, 70, 90 V/V%) were freeze-dried using a LABOR-MIM equipment for 13 hours. The temperature of the equipment and the samples was 60°C and -20 to 30°C respectively. Before lyophilization the ethanol content of the tinctures was evaporated.

2.2.3. EXTRACTION WITH METHANOL

0.1000 g dried and powdered plant sample was extracted twice with 20 ml methanol containing 0.05 M hydrochloric acid by sonication at 27 ± 2°C (Braun Labsonic U, Melsungen, Germany) for 2 x 10 min. The suspension was centrifugated at 6000 rpm (2500 g) for 10 min than was evaporated to dryness under reduced pressure (Rotavapor R-200, Büchi, Flawil, Switzerland) below 60°C. After evaporation samples were dissolved in 1,25 ml methanol containing 0.05 M hydrochloric acid, then diluted with 3,75 ml 0,05 M n-heptanesulfonic acid (HS) aqueous solution and purified by using solid-phase extraction.
2.2.4. EXTRACTION USED IN PHARMACOPOEIA

Powdered drug (0.500 g) was heated with 12 V/V% acetic acid (100 ml) for 30 min. using a cooled reflux condenser apparatus then the sample was cooled and filtered. The effluent was then alkalized with ammonia solution (pH = 8-9) and was subjected to ultrasonic bath extraction for 30 min in the presence of 100 ml chloroform (DAB 10- Method 1), 100 ml dichloromethane (Ph.Eur.5., Ph.Hg.VIII.-Method 2) and 100 ml n- buthanol (Method 3). The organic phase was dried using a reduced pressure evaporator apparatus and finally was dissolved in 3 ml methanol.

2.3. Phytochemical methods

2.3.1 QUANTITATIVE MEASUREMENT

- The total alkaloid content determination was based on the 10th German (DAB10), 5th European (Ph.Eur.5.) and the VIIIth Hungarian (Ph.Hg.VIII.) Pharmacopoeia using spectrophotometric measurements (570 nm) after complex formation with chromortopic acid.

2.3.2. CHROMATOGRAPHIC METHODS

- Thin layer chromatography – Kieselgel 60 F254 (Merck 0,2 mm), 10 x 20 cm. Eluent: formic acid : water : n-propanol (1 : 9 : 90 v/v/v); detection: the untreated plates was detected with UV light (254 + 365 nm) or developed with Dragendorff reagent.
- TLC-densitometry and biological tests - Kieselgel 60 F254, 10 x 20 cm; Eluent: dichloromethane : methanol (97 : 3 V/V) and chloroform : methanol (60 : 30 V/V); densitometer: IBM computer controlled Simadzu CS-9301PC (Kyoto, Japan).
- HPLC analysis – equipment: Surveyor (Thermo Finnigan, San Jose, CA, USA), column: Phenomenex Luna 5 µm C18 (250 mm x 4.6 mm i.d., Torrance, CA, USA), eluent: acetonitrile : methanol : 30 mM ammonium formate (14.7 : 18.67.3 v/v/v), flow rate: 1ml/min, detection: 280 mn.

2.3.3. MINERAL ELEMENT CONTENT

- ICP-OES (Thermo Jarrell Ash, spectrometer: Atom Scan 25, generator 2 kW, 27.124MHz, determined elements: Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Ti, V, Zn).

2.4 Antibacterial assessment with BioArena assay

Dried chromatographic plates were immersed into the bacterial suspension (Pseudomonas savastanoi pv. phaseolicola, London University Wye, UK; culture medium: King’s B broth; optical density: 0,7 at 560 nm; 1,5x10⁹ cell / ml) for 20 sec then for 2 hours in a chamber at 100% relative humidity at 28°C. After incubation plates were visualised by staining with the aqueous solution of 80 mg MMT (3-[4,5-dimethyl-tiazolyl-2]-2,5-diphenyl- tetrazolium bromide) and 100 mg Triton X-100 for 5 min. The dried and stained plates were incubated in a chamber at 100% relative humidity for 2 to 18 hours. Densitometric analysis was used to confirm the antibiotic effect of the alkaloid components at 305 and 590 nm respectively.

To investigate the mechanism of reaction of Chelidonium alkaloids we added the co-factors L-arginine (2,5 mg/ml), glutathione (2,5 mg/ml), and cooper(II)-sulfate (4,6,8 mg/100ml) to the cell suspension.
3. RESULTS AND CONCLUSIONS

3.1. Investigation of Chelidonii herba according to the Hungarian Pharmacopoeia (Ph. Hg. VIII.)

One of the critical points of safe application of medicinal herbs and their products is the quality of the basic substance. Application of *Chelidonium* drugs derived from the collection always has a higher risk than those of the cultivated drug basic substance. For this purpose we rated the herbal samples (Herb A and B) derived from our own collection in accordance with the paragraph related to Chelidonii herba in the Hungarian Pharmacopoeia (Ph. Hg. VIII.). After the micro- and macro-morphological analysis the required investigation for chemical impurities, weight-loss during exsiccation, total ash-content of the drug, qualitative (TLC) and the required quantitative (alkaloid content) analysis of the active ingredients were accomplished.

Excluding the total alkaloid content, the drugs met all the requirements of the related paragraph. However, according to the determination of the alkaloid content, none of the drugs achieved the value specified in the Hungarian Pharmacopoeia (Herb A = 0.53%; Herb B = 0.42%).

3.2 Critical evaluation of the total alkaloid content determination and altering the method

Due to the concurrent presence of Chelidonii herba alkaloids with a considerable different basis level, we investigated more precisely the role of the organic diluent used for changing the phases during the spectrophotometry total alkaloid assessment. Applying the official alkaloid content determination method described in the pharmacopoeia, we compared the same methods using different diluents in the liquid-liquid extraction.

It has been established that while according to the Ph.Hg. VIII. / Ph.Eur.5. (Method 2) a higher alkaloid concentrate was measured compared to the DAB 10 method (Method 1), still considerable amount of alkaloid remained unmeasured in the aqueous phase. For the
purpose of a more precise determination of the total alkaloid content we recommended the application of a more polar organic diluent, the n-butanol during the liquid-liquid extraction (Method 3. In the spectrophotometric method the reason for the application of n-butanol for changing the phases was the more effective extraction of coptisine, an alkaloid, which can be found in the highest amount in the herb according to the control HPLC assessments.

3.3. Investigation of the plant parts

3.3.1. DETERMINATION OF TOTAL ALKALOID CONTENTS IN THE PLANT PARTS

Production of a quality drug may be basically influenced by the proportion of plant parts. From the aspects of the synthesis, translocation and accumulation dynamics of the active agent, separate investigation of each individual plant part is also an important issue. Investigating the proportion of the total alkaloid content per plant organs in the plant samples, it has been established that generative organs (1,54%) contain the highest amount of active agent followed by the root (1,43%) with a slight difference. Alkaloid content of the leaf (0,71%) and stem (0,68%) were much lower than that of the generative organs.

3.3.2. DEVELOPMENT OF TLC-DENSITOMETRY METHOD FOR THE DETERMINATION OF ALKALOID COMPOSITION

Although HPLC methods are very precise, they are laboursome and time-consuming; therefore less suitable to serial-investigation of numerous samples. For the above reason we decided to develop a TLC-densitometry method for the quantitative determination of the principal alkaloids found in the root, leaf, stem and the generative organs of the greater celandine.

Two developing systems provided the solution for a thin-layer chromatography separation suitable for the densitometric determination of the five different alkaloids. The coptisine and berberine alkaloid components showed a proper separation when applying chloroform-methanol (60 : 30 v/v) as mobile phase, but the other three alkaloids migrated with the
solvent front. For the separation of the alkaloids chelidonine, chelerythrine and sanguinarine, methylene chloride-methanol (97 : 3 v/v) proved to be a suitable development system; in this case coptisine and berberine remained at the start.

Fluorescence mode was found to be suitable for the resolution of quaternary alkaloids while the reflexion mode was used for the exact quantitative determination of tertiary alkaloids. After selecting the adequate method of detection we determined the optimal extinction wavelengths (chelidonine: 320 nm, sanguinarine: 330 nm, coptisine: 360 nm, berberine: 340 nm, chelerythrine: 286 nm). After development the colour-intensity of the spots of the compounds determined in fluorescence mode has been increased, improving the sensitivity of the investigation; therefore we performed densitometry for the developed layers after storing them in a dark place for 12 hours. When plotting the calibration curves, the correlation coefficients falling between 0.993 and 0.999 showed a clear linear correlation between the amount of alkaloids applied to the layer sheets and the measured areas.

According to our model studies, the minimal detectable amount was 1-1 ng in the case of coptisine and sanguinarine alkaloids and 2-2 ng in the case of chelerythrine and berberine compounds. 100 ng chelidonine is the minimum amount, which can be exactly determined in reflexion mode. An adequate reproducibility of the instrumental measurements was confirmed by the relative standard deviation values of 0.67 – 1.24% calculated from six times repeated densitometric analysis of a given spot. On the basis of the relative standard deviation values of the reproducibility measurements (3,3 – 4,8%), the method proved to be suitable for the determination of the main alkaloid components of Chelidonium samples. In order to obtain more confidence of the suitability of the TLC-densitometry method, we compared our results with the results derived from the HPLC method.

We found that the TLC-densitometry developed by us is suitable for a quick, low-cost, adequately sensitive, properly reproducible and sufficiently accurate routine measurement of a large amount of plant samples. Due to the fluorescence property of quaternary alkaloids providing a high sensitivity, the developed TLC-densitometry can serve as an alternative method beside HPLC for investigating the alkaloid composition of Chelidonium drugs and derived products.
3.3.3. MEASURING THE ALKALOID COMPONENTS OF PLANT PARTS

On the basis of the measurement results confirmed even with two different methods, we established that the main alkaloid of the aerial part is the coptisine (leaf: 509 mg/100g, stem: 289.3 mg/100g, generative organ: 970 mg/100g). In the leaf and generative organ other alkaloids are present only at very low concentration beyond coptisine, the main alkaloid component. The coptisine concentration measured in the stem is lower, but chelidonine (78.2 mg/100g) and sanguinarine (107.9 mg/100g) are also considerable components. In the root chelidonine (376.3 mg/100g) can be found in the highest amount, followed by a high concentration of sanguinarine (335.7 mg/100g) and the coptisine (277.3 mg/100g), but the chelerythrine (168.7 mg/100g) and berberine (90.7 mg/100g) contents of the underground organ are also considerable.

3.4 Investigation of the dissolution of *Chelidonium* alkaloids in traditional pharmaceutical forms

The active agent content in a certain pharmaceutical form is highly affected by the technology used for manufacturing the medicinal product. We investigated three different pharmaceutical forms taking the traditional administration routes of medicinal herbs into consideration.

Depending on the extraction process, we found significant differences in the total alkaloid contents (0.14 – 0.39%; w/w). The product containing the highest total concentration of alkaloids was the tincture produced using 90v/v% alcohol (0.39 g/100g). Dissolution of the alkaloids in different pharmaceutical forms varied between 33.2% (infusion) and 92.9% (90v/v% tincture). The dissolution values of tinctures (69.1 – 92.9%) demonstrate the good solubility of *Chelidonium* alkaloids in semi-polar solvents. Among the aqueous pharmaceutical forms, infusion had a higher alkaloid concentration (0.19 g/100g). In the case of aqueous pharmaceutical forms, applying a longer dissolution time (infusion) is more advantageous for the dissolution of alkaloids, than applying a higher temperature (decoction).
As the main alkaloid component of the traditional products derived from the herb, we identified coptisine, which was present in different amounts in aqueous and alcoholic extracts (126.5 – 354.1 mg/100g). On the basis of the dissolution values, we established that coptisine dissolved in the highest amount (98%) in the 90 v/v% tincture. Tertiary chelidonine (79.9 – 91.9%) and protopine (80.2 – 94.2%) and the quaternary berberine (80.5 – 85.7%) also show a good dissolution in tinctures. However sanguinarine could be dissolved only in 54.4% in the case of tincture 90v/v%. Coptisine shows a 37% better dissolution in tincture of 90v/v% than 40v/v%. In 90v/v% tinctures the concentration of dissolved sanguinarine was two times higher than that in 40v/v% tincture.

The alkaloid content in aqueous extracts (decoction and infusion) is lower. Preparing a decoction was a more effective method for dissolution in the case of all five alkaloid compounds investigated by us. The dissolution values of chelidonine alkaloids in aqueous extracts show a less significant difference. Consequently, the dissolution time and applied temperature has less effect on the dissolution of chelidonine (decoction: 45.3%; infusion: 47.9%) and protopine (decoction: 46.7%; infusion: 50.9%) alkaloids than that of quaternary coptisine (decoction: 35.0%; infusion: 47.5%) and berberine (decoction: 36.1%; infusion: 48.8%). The dissolution value of sanguinarine was inconsiderable low in aqueous extracts (decoction: 2.7%; infusion: 5.1%).

The dissolution values of quaternary alkaloids (ionic state) in aqueous extracts were less than expected, which can be explained by their large hydrophobic molecular configuration. The inconsiderable low value of sanguinarine dissolution in aqueous, pH neutral tinctures could be explained by pseudo-base (carbinolamine) forming, which is known from the literature. This configuration appears only at higher pH values in the case of coptisine and berberine alkaloids.

The aim of our study on the dissolution in aqueous and alcoholic extracts was to provide data on the alkaloid composition, which is important both from the aspects of pharmaceutical and therapeutic quality.
3.5. Investigation of the mineral element content of Chelidonium drugs and traditional extracts

Since during our studies on antimicrobial activity we raised the possibility of an effect-modulating role of the micro- and macroelements found in the herb, and we did not find a detailed specification of mineral elements content of the greater celandine in the literature, thus we accomplished a modern ICP-OES analysis of *Chelidonium* drugs and herbal extracts prepared according to the requirements of traditional medicine manufacturing. As expected, the concentration of mineral elements in aqueous extracts usually exceeded the amounts measured in alcohol tinctures. The highest mineral element concentration was measured in decoctions. The dissolution values of aqueous extracts varied between 10% - 65%, whereas in tinctures values between 1.3% and 58.1% could be measured. In the best extraction method potassium (35.7% - 64.9%) and phosphorus (29% - 58.1%) were present. Potassium dissolves most efficiently in the infusion (64.9%) whereas the phosphorus in the tincture 40v/v% (58.1%). Aluminium, copper, iron, natrium and titan are present as hydrophobic compounds; the dissolution value of magnesium, sulphur and zinc is 20-40%. We established that the dissolution of mineral elements in tinctures decreases with an increasing alcohol concentration.

Evaluating the results from the aspect of safe therapeutic application, we established that neither the extracts prepared from the aerial part and the root of the greater celandine nor those prepared from the herba contain detectable toxic elements if prepared according to the rules of traditional medicine manufacturing.

The results of the dissolution studies on the extracts’ alkaloid and mineral element contents could be useful for a more established application of Chelidonii herba and for drug development.
3.6 Change in the total alkaloid content and alkaloid composition of the greater celandine during the vegetative period

Due to the inaccurate collection time specified in the monographies and the heterogenicity and contradiction of the data available in the literature, the aim of our further investigations was to determine the optimal time for collecting the Chelidonii herba and radix.

According to our studies, all herba drugs that were collected from two different places between 20 May and 10 October met all the requirements of the Hungarian Pharmacopoeia (total alkaloid content: 0.65-1.06%), however, the total alkaloid content of herbs showing a full blossoming on 30 April, was below the requirements (0.46-0.52%) specified in the pharmacopoeia (0.6%).

In the drugs derived from the herb and the radix collected on 20 July in Budapest a significant total alkaloid content was measured (herb: 1.06%, root: 1.71%). Alkaloid concentration in herbal drugs was decreased whereas that of in root drugs was increased in case of plants collected after 20 September. In the herb and root samples that were collected during the vegetative period the total alkaloid content and the change of each alkaloid component were also followed up. The highest coptisine concentration (840.5 mg/100g) was measured in the herba samples collected on 20 July. In the samples of the aerial parts the chelidonine (33.6 – 275.3 mg/100g) and sanguinarine (61.2 – 171.4 mg/100g) concentration was relatively high, while that of the protopine (29.1-80.7 mg/100g) and berberine (16.8 – 51.3 mg/100g) was lower.

The highest chelidonine concentration in the root – similarly to the herba – was found in the samples collected on 20 July (1247.2 mg/100g). Concentrations of chelidonine (254.9 – 537.8 mg/100g) and sanguinarine (216.1 – 475.3 mg/100g) simultaneously increased during the first blooming and crop maturation. A simultaneous increase is also observable during the second blooming, however the measured chelidonine (897.7 – 1136.2 mg/100g)content (897.7 – 1136.2 mg/100g) highly exceeds the sanguinarine content (437.1 – 557.1 mg/100g). The total alkaloid content and the alkaloid proportions relevant from the aspect of the therapeutic effect provide useful data also confirmed by modern chromatography for choosing the appropriate collection time. The highest total
alkaloid concentration was measured in the samples collected during the rest period, which is appropriate for the collection of both the herba and the root.

3.7. Investigation of the antibacterial activity of Chelidonium alkaloids with BioArena system

The frequent application of antibiotics increased the number of resistant microbial strains, thus protection technology studies investigating the antimicrobial activity of natural active agents come worldwide increasingly to the prominence. The antiviral, antibacterial and antifungal activity of Chelidonium alkaloids and extracts are widely known in the literature. The isoquinoline alkaloids of the greater celandine are N and O-methylated and these groups can be easily detached from them, so these molecules can be deemed as potential sources of formaldehyde, and may play a role in the mechanism of action of Chelidonium alkaloids through methylating and demethylating processes. An advanced method of the conventional bioautography, the BioArena system was used for studying the antibacterial activity of Chelidonium alkaloids.

The chelidonine, chelerythrine, sanguinarine, berberine and coptisine alkaloids of the greater celandine have an intensive antibacterial activity against Pseudomonas savastanoi pv. phaseolicola bacterial cells. After adding HCHO capturing molecules (L-arginine és glutathione) to the bacterial suspension we found a reduction in the antibacterial activity. According to our observations, 2 hours after the staining, L-arginine had a weaker, whereas glutathione a stronger effect on the reduction of the antibacterial effect. 18 hours after the staining, the HCHO-scavenger activity of L-arginine was reduced and the intensive antibacterial activity of the alkaloids developed again, however, glutathione almost completely eliminated the antibiotic activity of the alkaloids.

Our biological studies confirmed the antibacterial activity of Chelidonium alkaloids and support the assumption that the mechanism of action is a formaldehyde release due to demethylation.
5. PUBLICATIONS

In the topics of the thesis:


Partially in the topics of the thesis:


Citable abstracts:


Abstracts

Then, M., Szentmihályi, K., Sárközi, Á., Illés, V., Forgács, E.: Phytochemical extraction of different solvents of Chelidonium majus L. (Traditional and supercritical methods), The 9th Symposium on Handling of Environment and Biological Samples in Chromatography, October 10-13, 1999, Porto, Portugal.


Sárközi, Á., Then, M., Illés, V., Szentmihályi, K.: Comparative study on the supercritical fluid and microwave extraction of greater celandine (Chelidonium majus L.), 7th Meeting on Supercritical Fluids, December 6-8, 2000, Antibes, France.


Sárközi Á., Then M., Szentmihályi K., Szőke, É.: Comparing the element content of teas and tinctures obtained from Chelidonii herba, Semmelweis Symposium, November 6-7, 2003, Budapest.


