STUDIES REGARDING THE CHROMATOGRAPHIC SEPARATION OF SOME *CHELIDONIUM MAJUS* L. ALKALOIDS USING DIFFERENT SOLVENT SYSTEMS

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Abstract: Our study focuses on the investigation of a methanolic extract obtained from the roots of *Chelidonium majus* L. using the thin layer chromatography and different solvent systems in order to obtain the better resolution. The mobile phases that we used were represented by volumetric mixtures of methanol: chloroform: water (51:42:7), chloroform: methanol (7:3), chloroform: methanol (7:3) saturated with water, methanol: water (9:1), methanol: water (7,5:2,5), methyl-ethyl-ketone: water: methanol (67:30:3), chloroform: ethanol (35:1), chloroform: acetone: ethanol: toluene (10:5:5:5), 0,1% hydrochloric acid, chloroform: methanol: water (26:14:3) and n-propanol: water: formic acid (90:9:1). The last experiment was represented by a two dimensions thin layer chromatography with methanol: chloroform: water (51:42:7) as first dimension mobile phase and butanol: concentrated acetic acid: water (10:1:3) as second dimension mobile phase. The better resolution was obtained using the methanol: water (7,5:2,5), the methyl-ethyl-ketone: water: methanol (67:30:3) and the n-propanol: water: formic acid (90:9:1) mobile phases for one dimension TLC. The two dimensions TLC shows the separation of a big number of compounds that normally are not separated.

INTRODUCTION

The greater celandine is an herbaceous plant from the *Papaveraceae* family commonly found in North America, Europe and Asia. The plant has been used since ancient times in medical practice because of its pharmacological applications, due to the presence of many biologically active compounds, including alkaloids. The main constituents are isoquinoline alkaloids, belonging to different classes, such as benzophenanthridine (sanguinarine, chelidonine, and chelerithrine), protoberberine (berberine, coptisine) and protopine (protopine, allocryptopine) (Kursinszki *et al.*, 2006). Scientific data reveals the existence of 20 to 30 alkaloids, depending on the extraction and isolation methods that are used. The roots generally contain up to 2-3% alkaloids, while the aerial parts only 0,5-1,5% (Taborska *et al.*, 1994, Wichtl and Bisset, 1994). Although all the parts of the plant have been studied and medicinally used, most clinical trials have used the above-ground parts of the plant, usually collected at blooming time (Weiss, 1985).

The alkaloids qualitative and quantitative composition may vary, according to plant growth and geographical location (Bulatov *et al.*, 1990). The determination of the alkaloid content of various parts of the plant showed that coptisine represents 0,33% of the roots, 0,59% of the rhizomes and 1,07% of the leaves. Chelidonine is found to be 1,14% in the roots, 1,28% in the rhizomes and 0,07% in the leaves. The third important alkaloid is chelerythrine with a percentage of 0,77% in the roots, 1,06% in the rhizomes and 0,04% in the leaves. Berberine, sanguinarine and protopine are less represented (Fulde and Wichtl, 1994).

Our study consists in the investigation using the thin layer chromatography of a methanolic extract obtained from the roots of *Chelidonium majus* L. We focused on the analysis of different solvent systems in order to obtain the better resolution for future determinations.

MATERIALS AND METHODS

The plant material was collected from the Botanical Garden of Iasi in June 2010. The roots were separated from the rest of the plant and grounded. One gram of fresh root was mixed with 10 mL of acidulated methanol in the ratio methanol: concentrated HCl 100: 0,1 (*v/v*) and the cells were completely broken by ultrasonic treatment over the course of one hour. The resulting extract was centrifuged for 30 minutes at 5000 rpm and the supernatant was tested using different solvent systems and ascending thin layer chromatography.

The chromatographic material was represented by 20 x 20 cm Aluminium sheets with TLC Silica gel 60 provided by *Merck Germany*. All the solvents used for the preparation of the mobile phases have analytical purity. The method presents several steps. The first step consists in pre-saturate the chromatographic chamber with the solvent vapor during the night. The next steps are represented by spotting 10 µl of *C. majus* extract on a small area of the starting line,

perfectly drying the plate, the ascending migration, air-drying and UV-visualization. Because of the natural UV-fluorescence of *C. majus* alkaloids, no fluorescent indicators are needed. The photos were taken with a Canon A510 photo camera and using a MacroVue UV-VIS 20 transluminator. The retention factor (Rf) for each obtained spot was calculated.

RESULTS AND DISCUSSIONS

Because of the multitude of biologically active compounds, *Chelidonium majus* L. was the object of intensive biochemical, pharmaceutical and clinical studies during the last century when a multitude of alkaloids were exhaustively described. Despite the popularity of this plant in popular medicine, for many decades the scientists have showed themselves cautious regarding to his extensive use. There are many studies concerning the toxic potential of the greater celandine and especially his hepatotoxic action (Crijns *et al.*, 2002). For the other hand, a big number of studies confirm the important role that *C. majus* might have in cancer therapy (Lanvers-Kaminsky *et al.*, 2006), in inflammatory, respiratory and bacterial diseases (Tong *et al.*, 2001).

They are many data available concerning the extraction, separation and purification of *Chelidonium majus* L. alkaloids. Generally, isoquinoline alkaloids are extracted into alcoholic mixtures (ethanol or methanol) and quaternary and nonquaternary alkaloids are subsequently isolated by acidification and basification of the alcoholic residue (Bugatti *et al.*, 1991). Also, a big number of studies have been published dealing with the qualitative and quantitative analysis of isoquinoline alkaloids by different chromatographic techniques such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC), partition chromatography etc. (Qian *et al.*, 2010).

This study focuses on the investigation of different mobile phases in order to determine witch gives the better chromatographic resolution. With the purpose to eliminate the contamination with other compounds such as chlorophyll, we decide to use only the roots for our research. The acidification of the extract has as purpose the hydrolysis of the alkaloids from the glucidic component.

According to Verpoorte and Baerheim-Svendsen, 1983, the UV-colors for the main *C. majus* alkaloids are orange (chelidonine and protopine) and different varieties of yellow (berberine, chelerythrine, coptisine).

The first system used was represented by methanol, chloroform and water in a ratio of 51:42:7 (*v/v/v*). We obtained six spots: the first appears to be blue, with Rf = 0,29, the second one is week yellow - green (Rf = 0,42), the third is weak blue (Rf = 0,61), the fourth is bright green (Rf = 0,72), the fifth is dark green (Rf = 0,8) and the last one is intense orange (Rf = 0.95) (fig. 1).

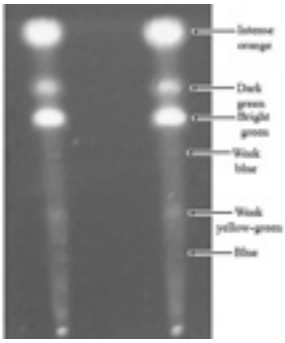

Fig. 1. TLC using methanol, chloroform and water (51:42:7 *v/v/v*) as mobile phase

We repeated the experiment with the mobile phase composed of chloroform and methanol 7:3 (*v/v*). The result was represented in figure 2 and consists in four spots: blue (Rf = 0,1), bright green (Rf = 0,4), dark green (Rf = 0,56) and intense orange (Rf = 0,91). The system resolution was less important then in the first case. We also believe that in both cases, the last intense orange spot contains more that one alkaloid, better methods of separation being needed.

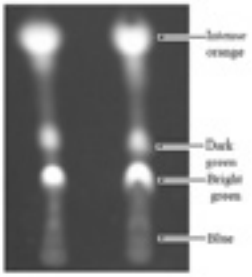

Fig. 2. TLC using chloroform and methanol (7:3 *v/v*) as mobile phase

The next system used was a mixture of chloroform and methanol in the same ratio (7:3 *v/v*), but saturated with water. We obtained nine spots as the figure 3 shows: blue (remains in the starting point), bright green (Rf = 0,06), dark green (Rf = 0,09), weak orange (Rf = 0,17), weak orange (Rf = 0,24), weak green (Rf = 0,3), orange (Rf = 0,55), bright yellow (Rf = 0,7), and intense orange (Rf = 0.8). Unlike the other two systems, the first three alkaloids or alkaloid mixtures were less separated.

According to Sárközi *et al.*, 2006, when chloroform and methanol 6:3 (*v/v*) was used, coptisine and berberine were separated, but chelidonine, chelerythrine and sanguinarine migrated with the solvent front. In the first three cases, we observed the appearance of very intense orange-yellow spots at the finishing line, using very similar mobile phases. These spots may be represented by the three appointed alkaloids. Using the water saturated mobile phase, the last spots were orange and yellow, which may suggest the separation of chelidonine, chelerythrine and sanguinarine.

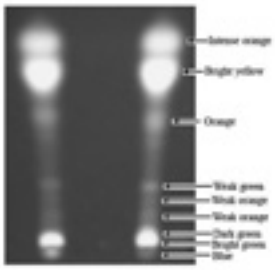

Fig. 3. TLC using a mobile phase composed of chloroform and methanol (7:3 *v/v*) saturated with water

The next system used consists in methanol and water (9:1 *v/v*). As the figure 4 shows, we obtained seven spots: bright yellow (in the starting point), bright green (Rf = 0,13), bright blue (Rf = 0,18), bright green (Rf = 0,22), dark green (Rf = 0,3), yellow (Rf = 0,64) and orange (Rf = 0,71). The second spot may be an alkaloid or a trace of the fourth one (the spots have identical color). We can conclude that the green spots that generally appear may contain more than one alkaloid and the use of this system leads to a better separation.

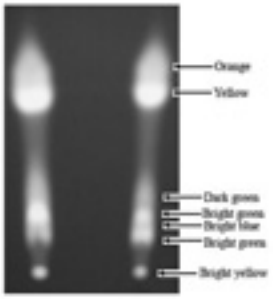

Fig. 4. TLC using methanol and water (9:1 *v/v*) as mobile phase

For the next determination we choose the same chemicals, but in another proportion (methanol: water 7,5:2,5 *v/v*). Analyzing the results (fig. 5) we observe the appearance of seven spots as follows: yellow (in the starting point), bright green (Rf = 0,22), bright blue (Rf = 0,32), dark green (Rf = 0,44), bright yellow (Rf = 0,56), orange (Rf = 0,64) and grey-orange (Rf = 0,77). Considering the color of the last spot, we assume that it may represent a trace of the previous one. Comparing with figure 4, we observe a better separation of the blue and green compounds using this system.

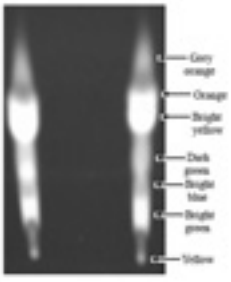

Fig. 5. TLC using a mobile phase composed of methanol and water 7,5:2,5 (*v/v*)

Another system used was represented by methyl-ethyl-ketone, water and methanol in the ratio of 67:30:3 (*v/v/v*). As figure 6 shows, we obtained eight spots: intense blue (Rf = 0,07), bright green (Rf = 0,17), dark green (Rf = 0,21), weak green (Rf = 0,31), intense yellow (Rf = 0,41), brown-red (Rf = 0,57), intense orange (Rf = 0,71) and orange (Rf = 0,78). This system seems to give the better resolution compared to the others previously analyzed. The novelty is represented by the appearance of the brown-red spot, compound probably very similar in structure with the orange one, fact that makes them difficult to separate. The last spot may be a separate compound or a trace of the previously one.

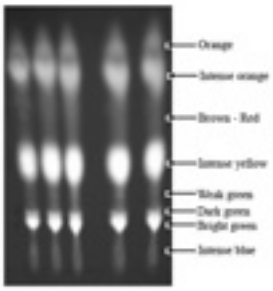

Fig. 6. TLC using a mobile phase composed of methyl-ethyl-ketone, water and methanol in the ratio of 67:30:3 (*v/v/v*)

The number of spots obtained using the mobile phase composed of chloroform and 96% ethanol in the ratio 35:1 (*v/v*) was five, as follows: green (in the starting point), yellow (Rf = 0,15), orange (Rf = 0,42), blue (Rf = 0,58) and weak orange (Rf = 0,68) (fig. 7). Unlike all the other cases, the component colored in blue seems to migrate more quickly using these system. Analyzing the results we may observe that the obtained resolution is not optimal.

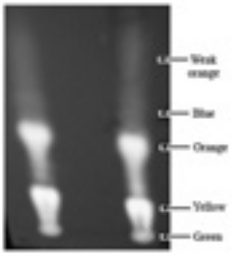

Fig. 7. TLC using chloroform and ethanol 35:1 (*v/v*) as mobile phase

For the next determination we choose a system composed of chloroform, acetone, 96% ethanol and toluene (10:5:5:5 *v/v/v/v*). This system separates six spots: green (in the starting point), yellow (Rf = 0,06), orange (Rf = 0,3), blue (Rf = 0,47), yellow-green (Rf = 0,59) and weak orange (0,7). The blue compound also migrates faster and the appearance of the last two spots indicates a better separation of the initially intense orange mixture. Nevertheless, this mobile phase can not be chosen for future investigations because of the possibility that some compounds may remain anchored at the starting point (fig. 8).

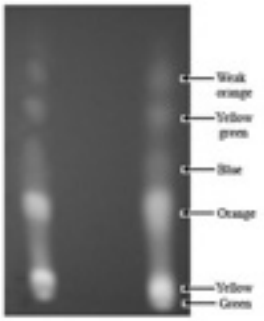

Fig. 8. TLC using a mobile phase composed of chloroform, acetone, ethanol and toluene 10:5:5:5 (*v/v/v/v*)

The mobile phase represented by 0,1% hydrochloric acid lead to the appearance of only two spots (fig. 9). The first is strong yellow (at the starting point) and we believe that contains the majority of the *Chelidonium majus* alkaloids and the last is blue with the retention factor 0,071.

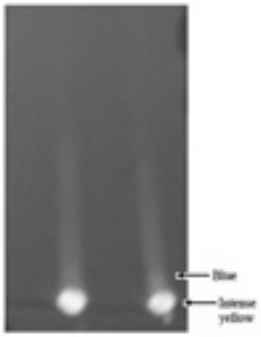

Fig. 9. TLC using 0,1% hydrochloric acid as mobile phase

Using a system composed of chloroform, methanol and water in the ratio 26:14:3 (*v/v/v*) (Artamonova and Kurkin, 2008) we obtained a number of eight spots (fig. 10): grey (Rf = 0,25), blue (Rf = 0,337), weak green (Rf = 0,42), brown (Rf = 0,51), grey-blue (Rf = 0,59), bright green (Rf = 0,64), dark green (Rf = 0,8) and yellow-orange (Rf = 0,95). This mobile phase confers a good resolution although we consider that the concentration of the last spot at the finishing line may suggest the existence of more than one alkaloid.

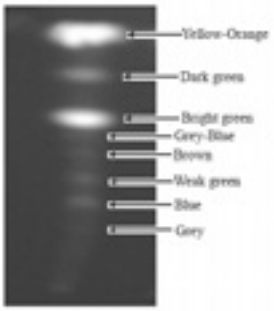

Fig. 10. TLC using a mobile phase composed of chloroform, methanol and water 26:14:3 (*v/v/v*)

The next system used is represented by n-propanol, water and formic acid in the ratio 90:9:1 (*v/v/v*). The use of these mobile phase leads to obtaining ten spots (fig. 11): green (in the starting point), blue (Rf = 0,064), brown (Rf = 0,1), yellow-green (Rf = 0,15), orange (Rf = 0,2), yellow-green (Rf = 0,26), green (Rf = 0,46), grey (Rf = 0,57), blue (Rf = 0,63) and yellow-orange (Rf = 0,71). This system seems to give the better resolution and the closely related alkaloids are better separated. This conclusion came from the fact that colors usually found in a singular manner, in this case can be identified in several places. According to Wagner *et al.*, 2009, using an identical mobile phase, the retention factor for coptisine was 0,15, for sanguinarine 0,3 – 0,4 and for chelidonine 0,75. Those three alkaloids generally appear colored in yellow-orange. For this reasons, we believe that the fourth spot may be represented by coptisine, the sixth by sanguinarine and finally, the last spot may be represented by chelidonine.

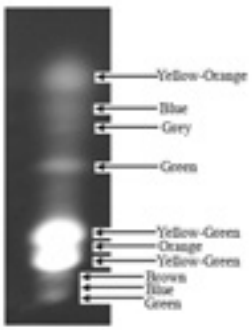

Fig. 11. TLC using a mobile phase composed of n-propanol, water and formic acid 90:9:1 (*v/v/v*)

The final experiment consists in a two dimensions thin layer chromatography. The mobile phase used for the first dimension is represented by methanol, chloroform and water (51:42:7 *v/v/v*) and for the second dimension consists in n-buthanol, concentrated acetic acid and water (10:1:3 *v/v/v*). As figure 12 shows, the migration in the second dimension leads to the separation of a big number of compounds. The first spot (blue) presents a retention factor equal to 0,22 for the first dimension and to 0,15 for the second dimension. The second spot (weak green) have Rf_{1D} = 0,358 and Rf_{2D} = 0,19. The third spot (bright green) have Rf_{1D} = 0.7 and Rf_{2D}

= 0,3 and the fourth spot (dark green) have Rf_{1D} = 0,61 and Rf_{2D} = 0,44. The next six spots seem to derive from a unique spot appeared in the first dimension because of the retention factor Rf_{1D} = 0,74. These spots are colored as follows: intense orange (Rf_{2D} = 0,39), bright yellow (Rf_{2D} = 0,47), red (Rf_{2D} = 0,52), weak yellow (Rf_{2D} = 0,58), blue (Rf_{2D} = 0,77) and weak orange (Rf_{2D} = 0,84).

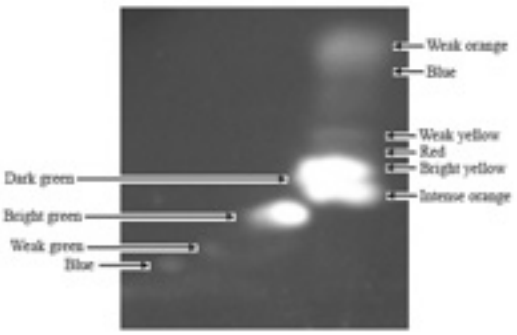

Fig. 12. Two dimensions TLC using a mobile phase composed of methanol, chloroform and water (51:42:7 *v/v/v*) for the first dimension and of n-buthanol, concentrated acetic acid and water (10:1:3 *v/v/v*) for the second dimension

The two dimensions TLC represent the better starting point for future investigations because of the separation of at least six compounds that in one dimension TLC appears to separate together in a big intense orange spot.

CONCLUSIONS

The mobile phases composed of chloroform and 96% ethanol in the ratio 35:1 (*v/v*), chloroform, acetone, 96% ethanol and toluene (10:5:5:5 *v/v/v/v*) and 0,1% hydrochloric acid can not be chosen for future investigations because of the possibility that some compounds may remain anchored at the starting point.

We observed a better separation of the compounds using the systems composed of methanol, chloroform and water 51:42:7 (*v/v/v*), chloroform and methanol 7:3 (*v/v*), chloroform and methanol (7:3 *v/v*), saturated with water and chloroform, methanol and water in the ratio 26:14:3 (*v/v/v*), although the intense orange spot that generally appear may contain more that one compound.

The use of methanol: water 9:1 (*v/v*) and methanol: water 7,5:2,5 (*v/v*) mobile phases leads to a better separation of the blue and green compounds.

The mobile phases composed of methyl-ethyl-ketone, water and methanol in the ratio of 67:30:3 (*v/v/v*) and n-propanol, water and formic acid in the ratio 90:9:1 (*v/v/v*) show the better one dimension TLC separation. In the first case, a brown-red spot, generally difficult to separate was observed. In the second case, closely related alkaloids are better separated.

Finally, the two dimensions TLC represents the better separation method because of the separation of at least six compounds that in other cases appear as a unique intense orange spot.

The previously described mobile phases will represent the object of future investigations using alkaloids standards for an appropriate identification.

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