Check for updates

NPC Natural Product Communications

2016
Vol. 11
No. 3
397 - 400

# Comparison of Seeds of *Colchicum speciosum* and *Gloriosa superba* in respect to Colchicine and Colchicoside Contents by RP-LC

**Mehmet Gumustas[a], Derya Çiçek Polat[b], Ceyda S. Kılıç[b*], Kemalettin Akalın[c], Sibel A. Ozkan[a] and Maksut Coşkun[b]**

[a]*Department of Analytical Chemistry*, *Faculty of Pharmacy*, *Ankara University*, *06100*, *Tandoğan*, *Ankara*, *Turkey*
[b]*Department of Pharmaceutical Botany*, *Faculty of Pharmacy*, *Ankara University*, *06100*, *Tandoğan*, *Ankara*, *Turkey*
[3]*Pharmaceutical Industry Association of Turkey*, *Ankara*, *Turkey*
*erdurak@pharmacy.ankara.edu.tr*

**Received: October 10th, 2015; Accepted: December 11th, 2015**

In this study, colchicine (CLN) and colchicoside (CLS) contents of the methanolic extracts of the seeds of *Colchicum speciosum* Steven that were collected from Uzungöl, Trabzon and also the seeds belonging to two different samples of *Gloriosa superba* Linn. imported from India were compared by using RP-LC (Reversed Phase High Pressure Liquid Chromatography). This proposed method is advantageous in terms of sample preparation and selective separation of the compounds. Also the method was successfully validated in accordance with the International Conference on Harmonization (ICH) guideline acceptance criteria for system suitability, linearity and range, precision, specificity and accuracy. As a conclusion of this analysis, the colchicoside and colchicine contents of *G. superba* (GSI), *G. superba* (GSII) and *C. speciosum* (CS) were found to be 312.9 mg/100 g and 333.1 mg/100 g; 434.0 mg/100 g and 471.1 mg/100 g, and 51.9 mg/100 g and 75.9 mg/100 g, respectively.

**Keywords:** HPLC, *Colchicum speciosum*, *Gloriosa superba*, Colchicine, Colchicoside.

*Colchicum speciosum* Steven and *Gloriosa superba* Linn. are perennial herbaceous plants belonging to the Liliaceae family [1, 2]. *C. speciosum* is native to the northern hemisphere, especially the Mediterranean. However, it is also distributed in Europe, North Africa and the north east part of East Himalayas [1, 3]. *C. speciosum* is native to the Eastern Black Sea Region within Turkey [4]. However, in recent literature, the genus has been placed within the Colchicaceae [5]. Corms, leaves, seeds and flowers of the species, along with other species found within the genus, contain the alkaloid colchicine [4]. The seeds of the species also contain 2-demethyl colchicine, 3-demethyl colchicine, colchicoside, demecolcine, 2-demethylcolcine, and 3-2-demethylcolcine [6-8].

*Gloriosa superba* Linn. grows naturally in Asia and Africa and is distributed from the Northwest Himalayas to Assam and the Deccan Islands [2], and is found in equatorial countries such as India and Sri Lanka [9-10]. The species is commonly known by the names flame lily, climbing lily, creeping lily, glory lily, gloriosa lily, tiger claw and fire lily [9]. Colchicine (CLN) is the major chemical marker of *G. superba* (0.7-0.9%), and lumicolchicine, 3-demethyl-*N*-deformyl-*N*-deacetylcolchicine, and 3-demethylcolchicine are also present [11]. It is known as a specific medication for gout and Familial Mediterranean Fever (FMF) [4], and is also used in other diseases like cancer and scrofula, and acts as an antipyretic, anthelmintic, purgative and antiabortive. It is used widely off-label by naturopaths for a number of treatments, including the treatment of back pain, and it is also used as an anti-inflammatory agent for the long-term treatment of Behcet's disease [12]. More recently, usage of CLN has been proposed for the treatment of pericarditis. Although clinicians are often skeptical of the possible use of CLN for this disease, growing evidence suggests that it may be useful in patients with recurrent pericarditis [13]. It is used in genetic studies [4], especially in biological and breeding studies to produce polyploids and in tubulin binding assays as a positive control since CLN has potent affinity for tubulin [14]. Though it can be used as an anticancer agent [4], CLN itself is too toxic for human use as an anti-tumor drug (animal cells are more sensitive to CLN than plant cells, and, therefore, it is usually lethal to dividing animal cells, even at low concentrations), therefore its derivatives are used [12]. However, plant cells can survive in a state of colchicine induced mitotic arrest (C-mitosis) for several days and return to division after CLN is removed [13].

CLN levels of *G. superba* corms have been reported to be around 0.9%. *G. superba* acts as a better source of commercial CLN than the *Colchicum* species. Concentration in *C. autumnale* is reported to be 0.3-0.5%. The seeds are the best source of CLN as their content is 2-5 times higher than that of the tubers [11]. In another study, the content of CLNs has been estimated to be 0.6% in seeds [15]. However comparison with *C. speciosum* growing naturally in Turkey has not been performed as far as we are aware.

Liquid chromatographic techniques, especially RP-LC, are used for the determination of compounds in natural products [16]. The advantage of RP-LC is the large choice of solid phases and the high number of plates. Difficulties have been encountered using HPTLC on pure silica (tailing, co-elution), and, although it is possible to try reverse phase HPTLC, this technique is more expensive and the number of reagents used in this method becomes more limited. From a regulatory point of view, HPTLC has not yet been accepted.

The aim of the present study was to compare the CLN and CLS contents of *C. speciosum* seeds that had been collected from Uzungol, Trabzon, Turkey by our team and the seeds belonging to two different, randomly selected samples of *G. superba* imported from India and to investigate whether or not *C. speciosum* seeds can be utilized as a productive source of colchicine and colchicoside. For this reason, a fully validated gradient RP-LC method was developed for the sensitive and simultaneous determination of CLN and CLS from these plant species. The proposed method was found

to be rapid, economical, precise, and accurate and it was also validated according to the International Conference on Harmonization (ICH) guidelines [17]. The details of the plants are given in Table 1. A voucher specimen of *C. speciosum* collected from Trabzon, Turkey is kept at AEF (Herbarium of Ankara University Faculty of Pharmacy). The samples of the seeds of *G. superba* were purchased from two random Indian companies and, therefore, herbarium specimens were not prepared.

**Table 1:** Details of the studied plants.

| Species | Collected/Imported from | Sample Code |
|---|---|---|
| *Gloriosa superba* Linn. | SRS Trust, India (2012) | GSI |
| *Gloriosa superba* | MSV IMPEX, India (2012) | GSII |
| *Colchicum speciosum* Steven | Trabzon; between Uzungöl and Lustra, 1200-200 m. altitude, (14.7.1993) | CS |

In this study, a comparison was made by HPLC of the methanolic extracts of the seeds of *C. speciosum* collected from Trabzon-Uzungöl and the seeds of two *G. superba* samples purchased from India with respect to their colchicine and colchicoside contents. The optimized HPLC method was validated in accordance with ICH guidelines from the view point of linearity, LOD, LOQ, precision, and accuracy. It also provided a simple, cost-effective, high throughput determination of related compounds from the plant materials.

The *G. superba* samples were shown to contain more CLN and CLS than *C. speciosum*, which is known to be rich in these active substances. However, the results for *C. speciosum* were lower than those obtained by Tanker *et al*. in 2000 on the seeds of the same species [18]. The lower yield that was encountered in this study may be due to the plant material being old, or it may be due to the differences in RP-LC conditions. Nevertheless, the previously obtained yields were also lower than the amounts found in the *Gloriosa* samples in this study. The results of the two *G. superba* samples were found to be parallel to the literature values [19, 20]. As a result, it was confirmed that *G. superba* is an important source of CLN and CLS. Even the waste material of this plant is utilized. The pericarp of the plant is an agricultural waste after the seeds have been used for medicinal properties. There is a potential application of the pericarp carbon for fast green dye removal due to its high availability as a low cost absorbent [21]. So the fruits of the plant can also be used after the extraction of active substances for medicinal purposes. However, since the plant is commercialized to a high extent, it faces the threat of extinction and is included in the Red Data Book as an endangered species. Therefore, measures should be taken to ensure the sustainability of this species [22-24].

One of these measures may be the exploration of different plant sources for colchicine and colchicoside. Obtained results showed that the content of these compounds is approximately one sixth that of randomly selected commercial *Gloriosa* samples. This might be considered as a significant disadvantage for the utilization of *C. speciosum* growing naturally in Turkey as a source of colchicine and colchicoside. However, unlike *G. superba, C. speciosum* is not classified as an endangered species according to the Red Data Book of Turkey [25]. Thus, despite the low content of these active substances in *C. speciosum*, the sustainability of the species may compensate for this low quantity in the long term and this species might be considered as an alternative source of colchicine and colchicoside in the future.

## Experimental

***General:*** CLN and CLS were supplied from Sigma-Aldrich (Istanbul, Turkey) and Carl Roth, (İstanbul, Turkey), respectively. All chemicals were of analytical grade and the solvents used for the RP-LC system were of chromatographic grade. They were employed without further purification. Methanol, potassium bromide, phosphoric acid (85%), sodium hydroxide, triethylamine, trifluoroacetic acid, and $CaCO_3$ were procured from Sigma-Aldrich (Munich, Germany). Doubly distilled water was used for preparing the mobile phase solutions. The mobile phase was filtered through 0.45 µm PTFE membranes using a vacuum pump and degassed before analysis.

The seeds (10 g) of *C. speciosum* and *G. superba* were ground, and subjected to Soxhlet extraction for 6 h with 200 mL of methanol and the addition of 0.15 g $CaCO_3$. The methanol extracts were evaporated in a rotary evaporator (Heidolph, Germany). Extract yields were 2.47 g, 2.59 g and 2.69 g for GSI, GSII and Cs, respectively.

***Instrumentation and chromatographic conditions:*** An integrated chromatographic system, Agilent 1100 series (Wilmington, DE) was used for the method development and validation studies. The gradient chromatographic separation of the bulk active pharmaceutical ingredients (API) and plant materials were performed using a binary solvent system consisting of A) methanol and B) water (containing triethylamine 0.1%, trifluoroacetic acid 0.1% (resultant pH 2.30)). The elution program is tabulated in Table 2.

**Table 2:** Timetable for gradient elution.

| Time* | A (%) | B (%) |
|---|---|---|
| 0.00 | 30 | 70 |
| 4.00 | 30 | 70 |
| 4.01 | 45 | 55 |
| 10.40 | 45 | 55 |
| 10.50 | 30 | 70 |

*The analysis was completed within 11 min and 3 min of post-time was used.

Separations were performed with a flow rate of 1.25 $mL.min^{-1}$ at 40°C by using an X Terra® RP-18 (250 mm x 4.60 mm ID, 5µm, Waters, Milford, MA, USA) stainless steel analytical stationary phase. Analysis was performed with the detection wavelength of 350 nm. The pH was measured using a pH meter Model 538 (WTW, Weilheim, Germany) using a combined electrode (glass electrode-reference electrode) with an accuracy of ±0.05 pH.

***Preparation of plant materials and solutions:*** 10 mg of each API was transferred to a volumetric flask, separately. 10 mL of methanol was added to the volumetric flasks up to the mark as a final concentration of 1 $mg.mL^{-1}$. The working standard solutions of appropriate concentration were prepared daily by diluting the stock standard solutions with methanol. The seeds were ground; 10 g of each plant, with the addition of 0.15 g $CaCO_3$, were subjected to Soxhlet extraction with 200 mL methanol for 6 h. The methanol extracts were evaporated in a rotary evaporator (Heidolph, Germany). Extract yields were 2.47 g, 2.58 g and 2.69 g for GSI, GSII and CS, respectively. All solutions were kept in the dark in a refrigerator and were used within 24 h to avoid decomposition.

***Validation of the method:*** Optimization of the method began with checking and calculating the system suitability parameters. After this step the optimized chromatographic conditions were validated by evaluating linearity and range, precision, limit of detection (LOD) and limit of quantification (LOQ), specificity, accuracy, robustness and the other parameters according to the ICH guideline Q2 R1 [17].

***Linearity and range:*** The linearity of response for the compounds was assessed by analysis of 5 repetitive levels of calibration curve in the range of 1-300 $µg.mL^{-1}$ for CLN and CLS. The relative peak areas were used to construct a curve of each component from which

its regression equation could be calculated. The detection limit (LOD) is the value that is the lowest amount of an analyte in a sample which can be detected, but not necessarily quantified. The quantification limit (LOQ) is the lowest amount of an analyte in a sample which can be quantitatively determined. LOD and LOQ were calculated from the equations of LOD=3.3 x s/m and LOQ=10 x s/m [17, 26] where *s* represents the standard deviation of response and *m* the slope of the calibration curve.

***Precision and accuracy:*** Precision was checked on the same day (n=5) and on 3 different days (n=5) over a period of one week. Precision was expressed as relative standard deviation (RSD %). The precision and accuracy are described in a quantitative fashion by the use of relative errors (Bias %). One example of a relative error is the accuracy, which describes the deviation from the expected results [17, 26]. The effect of small but deliberate variations in the method was also evaluated for showing the robustness of the proposed work. The flow rate, pH, mobile phase composition and wavelength were changed in smaller variations than the RSD% values with the peak areas measured.

***Optimization of chromatographic conditions:*** A gradient HPLC method coupled with diode array detection (DAD) was developed to provide a suitable procedure for the routine quality control analysis of a binary mixture of CLN and CLS. Development of a method for liquid chromatography is the key point for analysis. To reach this goal, the most important parameter is the achievement of sufficient resolution of the pharmaceutical target compounds from all other excipients and matrix effects that can be caused by plant material. On the other hand, the developed method should be completed within an applicable analysis time with acceptable efficiency. For this reason, there are some analytical conditions which have been developed in this step such as stationary phase type with the dimensions and particle sizes, composition of the mobile phase, column oven temperature, type and pH of the buffer.

For optimization of the stationary phase, different reversed phase columns were tested. The best separation with symmetrical and sharp peak shapes for these compounds were achieved by using the X Terra® RP-18 (250 mm x 4.60 mm ID, 5µm, Waters, Milford, MA, USA) stationary phase. Another step of optimization was to find the best organic solvent and arrange its composition with an aqueous buffer phase. For this reason, methanol and acetonitrile were evaluated using varied percentages of buffer solutions. Methanol was chosen instead of acetonitrile because of its economical and toxicological advantages, and it showed efficient separation and resolution. As a result of these experiments, capacity factor and matrix effect were observed to create some problems, following which the analysis was performed in gradient conditions and subsequently no interference with other compounds that originated from interferences and matrix were observed.

Different acidic and basic additives were used for the preparation of the buffer. After the addition of 0.1% trifluoroacetic acid and an equal amount of triethylamine, peak shapes of the target compounds improved. In order to adjust the analysis time, flow rate was arranged to 1.25 mL.min$^{-1}$. Temperature is another effect to consider for the optimization of separation. The column temperature was changed in the range from 25$^{o}$C to 45$^{o}$C. Finally, it was adjusted to 40$^{o}$C with a detection wavelength of 350 nm. The final optimized conditions approved with the system suitability test parameters are tabulated in Table 3. Using the optimized operating conditions, retention times were obtained of 3.70 min for CLS and 9.10 min for CLN with 0.09 and 0.08% relative standard deviations from 5 repetitive injections, respectively.

**Table 3:** System suitability test parameters of the compounds.

| Compounds | CLS | CLN | Recommended values |
|---|---|---|---|
| **Retention Time** | 3.88 | 9.61 | - |
| **RSD% of Retention Time** | 0.09 | 0.08 | <2.0 |
| **Capacity Factor (k)** | 0.68 | 3.33 | >1.0 |
| **Selectivity factor (α)** | - | 4.87 | >1.5 |
| **Resolution Factor (Rs)** | - | 17.43 | >1.5 |
| **Tailing (T)** | 0.88 | 1.18 | <2.0 |
| **Theoretical Plate Numbers (N)** | 2800 | 25104 | >2000 |

***Validation and determination of CLN and CLS:*** Detailed parameters of the RP-LC analysis are given in Tables 3 and 4. At the beginning of the validation studies, system suitability tests were performed. For this reason selectivity, capacity, resolution, tailing, and theoretical plate number were calculated. The obtained results showed that the optimized method is suitable for the validation (Table 3).

For linearity, a linear correlation was obtained between the peak areas and the prepared concentrations in the range between 1.00 to 300 µg.mL$^{-1}$, with regression equations y=14.53x+42.011 for CLS and y=18.96x+47.18 for CLN, with correlation coefficients greater than 0.999. LOD and LOQ results were calculated as mentioned before and LOD values were found to be 0.066 and 0.034 µg.mL$^{-1}$; LOQ values were calculated as 0.198 and 0.103 µg.mL$^{-1}$ for CLS and CLN respectively.

**Table 4:** Calibration values for CLS and CLN.

| | CLS | CLN |
|---|---|---|
| **Retention time** | 3.88 | 9.61 |
| **Linearity range (µg.mL$^{-1}$)** | 1-300 | 1-300 |
| **Slope** | 14.529 | 18.958 |
| **Intercept** | 42.011 | 47.182 |
| **Correlation coefficient** | 0.999 | 0.999 |
| **Standard error of the slope** | 1.728x10$^{-1}$ | 1.890x10$^{-1}$ |
| **Standard error of the intercept** | 2.499 | 2.733 |
| **Limit of detection** | 0.066 | 0.034 |
| **Limit of quantification** | 0.198 | 0.103 |
| **Repeatability (RSD%)** | 0.387 | 0.110 |
| **Reproducibility (RSD%)** | 0.461 | 0.218 |

To evaluate the quality of the method, precision and accuracy were determined. For the precision, the within day and between days reproducibility of retention times and peak areas were tested by 5 repeated injections of the selected concentrations. The values of both compounds are expressed in terms of RSD % values calculated from the data of inter day results. RSD % values were found to indicate good repeatability (Table 4). The intraday measurements showed the degree of reproducibility of the results obtained by analysis of the samples on different days. The samples of the selected concentration levels of CLS and CLN were analyzed at the same laboratory on 3 consecutive days by performing 5 measurements on each day. The obtained data were below 2.0% RSD (Table 4). The results indicated that the proposed method is equally precise both on the same day and on separate days.

**Table 5:** Analysis results of the plant material.

| **Species** | ***G. superba* I** | | ***G. superba* II** | | ***C. speciosum*** | |
|---|---|---|---|---|---|---|
| | **CLS** | **CLN** | **CLS** | **CLN** | **CLS** | **CLN** |
| **Amount found in extract (mg.100g$^{-1}$)** | 312.9 | 333.1 | 434.0 | 471.1 | 51.9 | 75.9 |
| **RSD (%)** | 1.13 | 0.74 | 0.29 | 0.56 | 0.52 | 0.21 |
| **Added (µg.mL$^{-1}$)** | 10 | 10 | 10 | 10 | 10 | 10 |
| **Found (µg.mL$^{-1}$)** | 10.38 | 10.57 | 10.10 | 10.41 | 9.64 | 9.97 |
| **Recovery (%)** | 103.8 | 105.7 | 101.2 | 104.1 | 96.4 | 99.7 |
| **RSD % of recovery** | 1.76 | 0.58 | 0.91 | 0.85 | 1.98 | 0.68 |
| **BIAS (%)** | 3.77 | 5.69 | 1.03 | 4.12 | 3.67 | 0.30 |

***Analysis of plant materials:*** For the injection of plant materials, the below specified process was used. A certain amount of plant extract was first dissolved in 10 mL of the mobile phase. One mL of this solution was diluted to 1.5 mL with the mobile phase, filtered

through syringe filters with a pore diameter of 0.45 µm (Minisart®, Sartorius, Ankara, Turkey) and 10 µL of this solution was injected into the HPLC device. As starting quantities, 0.1096 g, 0.0722 g and 0.1737 g extracts were taken from GSI, GSII and CS, respectively.

In order to know the applicability of the proposed methods, *C. speciosum* and *G. superba* containing CLS and CLN were studied using the previously optimized method (Table 5). Consequently, application of the RP-LC method to plant materials is possible after the simple steps of filtration and dilution. Five replicate determinations were made and repeatability results were found to be 0.39 % and 0.46 % for CLS and 0.11 % and 0.22 % for CLN, respectively. The obtained results appear precise in accordance with the ICH guideline (Table 5).

The accuracy of the method was examined by standard addition to the plant materials and the results were found to be 103.8% and 105.7 % for *G. superba* I, 101.2% and 104.1% for *G. superba* II and 96.4 % and 99.7 % for CLS and CLN, respectively. Bias% results are also tabulated in Table 5 and all results tabulated in Table 5 showed good precision and accuracy. Therefore it can be said that no interference with the main peaks of CLS and CLN was present.

## References


[1] Brickell CD. (**1984**) *Colchicum speciosum* Steven. In *Flora of Turkey and the Aegean Islands*, Vol ***8***, Davis PH. (Ed), Edinburgh University Press, Edinburgh. UK. 347-348.

[2] Kavina J, Gopi R, Panneerselvam R. (**2011**) *Gloriosa superba* Linn-Medicinally important plant. *Drug Invention Today*, ***3***, 69-71.

[3] Sütlüpınar N. (**1983**) Türkiye'nin Sonbaharda Çiçek Açan *Colchicum* Türleri Üzerinde Araştırmalar (Investigations on *Colchicum* species of Turkey flowering in autumn). *Doğabilim Dergisi*, *Temel Bilim*, ***7***, 355-359

[4] Akbulut S, Özkan ZC. (**2009**) Minimum leaf width as an indicator of *Colchicum speciosum* Steven (*Liliaceae*) suitable for collection. *Journal of Medicinal Plants Research*, ***3***, 377-381.

[5] Karakaş ÖM, Türktaş M, Ertuğrul F, Kaya E. (**2014**) Use of fluorescent-based amplified fragment length polymorphism to resolve phylogenetic relationships of *Colchicum* species from Turkey. *Genetics and Molecular Research*, ***13***, 1480-1490.

[6] Simanek V, Husek A, Vakla I, Sütlüpınar N. (**1990**) Phytochemical studies of Turkish *Colchicum* and *Merendera* species. *Herba Hungarica*, ***29***, 64-68.

[7] Kupchan SM, Britton RW, Chiang CK, Noyanalpan N, Ziegler MF. (**1973**) Tumor inhibitors. 88. The antileukemic principles of *Colchicum speciosum*. *Lloydia*, ***36***, 338-340.

[8] Khanam PS, Sangeetha B, Kumar BV, Kiran U, Priyadarshimi PI, Ram R, Sridhar MS, Kumar VS. (**2015**) *Gloriosa superba* ingestion: hair loss and acute renal failure. *Indian Journal of Nephrology*, ***25***, 174-176

[9] Goonerate IK, Weeratunga P, Caldera M, Gamage R. (**2014**) Toxic encephalopathy due to colchicine – *Gloriosa superba* poisoning. *Practical Neurology*, ***14***, 357-359.

[10] Srivastata S, Misra A, Kumar Shukla P, Kumar B, Lata S, Rawat KS. (**2014**) A validated over pressured layered chromatography (OPLC) method for separation and quantification of colchicine in *Gloriosa superba* (L.) tubers from different geographical regions. *RSC Advances*, ***4***, 60902-60906.

[11] Sapra S, Bhalla Y, Nandani Sharma S, Singh G, Nepali K, Budhiraja A, Dhar KL. (**2013**) Colchicine and its various physicochemical and biological aspects. *Medicinal Chemistry Research*, ***22***, 531-547.

[12] Imazio M, Brucato A, Trinchero R, Spodick D, Adler Y. (**2009**) Colchicine for pericarditis: hype or hope? *European Heart Journal*, ***30***, 532-539.

[13] Bharathi P, Philomina D, Chakkaravarthi S. (**2006**) Antimitotic effect of colchicine from six different species of *Gloriosa* in onion roots (*Allium cepa*). *Journal of Medical Sciences*, ***6***, 420-425.

[14] Labib S, Boujraf S, Harandou M. (**2014**) Fatal colchicine intoxication. *Saudi Journal of Anesthesia*, ***8***, 394-395.

[15] Jana S, Shekhawat GS. (**2011**) Critical review on medicinally potent plant species: *Gloriosa superba. Fitoterapia*, ***82***, 293-301.

[16] Gumustas M, Kurbanoglu S, Uslu B, Ozkan SA. (**2013**) UPLC versus HPLC on drug analysis: advantageous applications and their validation parameters. *Chromatographia*, ***76***, 1365-1427.

[17] ICH Guideline (Q2A) (R1) (**2005**) *Validation of analytical procedures: Text and methodology*. Geneva, Switzerland.

[18] Tanker M, Coşkun M, Özden S, Koyuncu M, Güvenç A, Soner O, Altun ML, Akalın K, Erdurak CS. (**2000**) Ülkemizde Yetişen *Colchicum* Türlerinden Tedavide Kullanılmak Amacıyla Standart *Colchicum* Tohumu Ekstrelerinin Hazırlanması ve Kolşisin Elde Edilmesi (Preparation of seed extracts from *Colchicum* species growing in our country and obtaining colchicine to be used in medicine. Ankara University Research Fund Project (Project No: 97-03-00-01), Ankara.

[19] Sonali J, Shekhawat GS. (**2011**) Critical review on medicinally potent plant species: *Gloriosa superba*. *Fitoterapia*, ***82***, 293-301.

[20] Kanan S, Daniel W, Ruba A, Rajalakshimi AR, Kumaragurubaran K. (**2007**) Optimization of solvent for effective isolation of colchicines from *Gloriosa superba* L. seeds. *Natural Product Research*, ***21***, 469-472.

[21] Ramalakshmi S, Muthuchelian K, Swaminathan K. (**2012**) Comparative studies on removal of fast green dye from aqueous solutions by activated carbon prepared from *Gloriosa superba* waste and *Alternaria raphanin* fungal biomass. *Journal of Environmental Science and Technology*, ***5***, 222-231.

[22] Singh D, Mishra M, Singh Yadav A. (**2012**) Callus induction from corm of *Gloriosa superba* Linn: An endangered medicinal plant. *BTAIJ (BioTechnology An Indian Journal)*, ***6***, 53-55.

[23] Singh D, Mishta M, Singh Yadav A. (**2013**) *Gloriosa superba* Linn: An important endangered medicinal plant and their conservation strategies, *International Journal of Botany (IJBR)*, ***3***, 19-26.

[24] Pant M, Purohit M. (**2013**) *Gloriosa superba* L.: An ethnomedicinal plant from Mandsaur district showing extinction in other parts of Madhya Pradesh. *International Journal of Medicinal Plants*, ***105***, 277-280.

[25] Ekim T, Koyuncu M, Vural M, Duman H, Aytaç Z, Adıgüzel N. **(2000)** In *Türkiye Bitkileri Kırmızı Kitabı - Eğrelti ve Tohumlu Bitkiler* (Red Data Book of Turkish Plants – Pteridophyta and Spermatophyta). Türkiye Tabiatını Koruma Derneği, Van Yüzüncü Yıl Üniversitesi, Ankara, Turkey.

[26] The United States Pharmacopeia (The USP 32-NF 27) The Official Compendia of Standards, United States Pharmacopeial Convention, MD, Rockville, USA (**2009**).