

Determination of Colchicine Contents in Different Jordanian *Colchicum* spp.

Walid Turk^{1*} and Suleiman Olimat²

¹ Faculty of Pharmacy, Middle East University, Amman, Jordan.

² Department of Pharmaceutical Sciences, Faculty of Pharmacy, The University of Jordan, Amman, Jordan.

ABSTRACT

Eight *Colchicum* spp. grown wild in Jordan have been collected from different parts of the country. The major component, colchicine alkaloid was, determined by using HPLC. *C. crocifolium* contains the highest percentage of colchicines

Keywords: *Colchicum* spp, colchicines, plants, Jordan.

1. INTRODUCTION

The genus *Colchicum* belongs to the family Colchicaceae, which comprises of 19 genera, and 225 species¹. Eight species of *Colchicum* are growing wild in Jordan, namely: *C. brachyphyllum* Boiss. & Haussk. ex Boiss., *C. crocifolium* Boiss, *C. heirosolymitanum* Feinbr, *C. tauri* Siehe ex Stefanov, *C. ritchii* R. Br., *C. stevenii* Kunth, *C. triphyllum* G. Kunze, and *C. tunicatum* Feinbr⁽²⁻⁴⁾.

The pharmacological activity of this genus is attributed to the presence of colchicinoids, mainly colchicine⁽⁵⁾; which was first isolated by Pelletier and Caventou in 1820⁽⁶⁾. Colchicine; (S)-N-(5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo-[a]heptalen-7-yl) acetamide⁽⁷⁻⁹⁾, occurs as yellowish-white amorphous scales with a very bad bitter taste, darkens on exposure to light^(8,9).

Colchicine, is still the drug of choice for treatment of gout⁽¹⁰⁾, and is used for the treatment of a number of pro-inflammatory disorders, such as familial Mediterranean fever⁽¹¹⁾, and Behcet's disease⁽¹²⁾. Clinical studies have proved colchicine to possess a potent anti-tumor activity. But its use as an anti-neoplastic drug is limited,

due to lack of tumor selectivity and high toxicity^(13,14).

Among all species of *Colchicum*, *C. autumnale* is the best source for colchicine^(8,14). The richest plant parts in colchicine are the corms and seeds; *C. autumnale*

seeds contain 0.6–1.2%, while corms contain up to about 0.6%⁽¹⁵⁾. Seeds are mainly used by the pharmaceutical industry for the extraction of colchicinoids⁽¹⁴⁾.

Plant Collection and Identification

Plant materials used in this study were collected from the following areas:

C. brachyphyllum from Tafila areas. *C. crocifolium* from Safawi area. *C. heirosolymitanum* from Sarw area (Salt). *C. tauri* from Alook area. *C. ritchii* from Petra area. *C. stevenii* from Hofa area. *C. triphyllum* from Wadi Rum area and *C. tunicatum* from Dana area. Corms of the different species were collected from October to December of 2010, in the mentioned area.

The collected material was identified by Prof. Daud El-Eisawi, Plant Taxonomists, Department of Biological Sciences, Faculty of Science, The University of Jordan. The plant raw material, corms, were cleaned and sliced into small pieces, and all were subjected to air drying at room temperature. After drying, exact weights were recorded. The corms were grounded to powder using a laboratory mill and then used for extraction.

* s.olimat@ju.edu.jo

Received on 25/1/2015 and Accepted for Publication on 20/4/2015.

Plant Material Extraction

The ground plant materials extracted with (0.5L) Methanol (MeOH 99%), was soaked at room temperature for 24 hr, and then the MeOH fraction was filtered. The

extraction procedure was repeated five times for each sample to ensure high recovery. Rotary Evaporator brought the filtrate to dryness (Table 1). The residue obtained was used for crude alkaloid isolation.

Table 1. The weight of the extract and the percentage of colchicine

	Plant	Weight of the plant (g)	Weight of the Extract (g)	Colchicine Conc 1 µg/mL
1	<i>Colchicum Hierosolymmitanum</i>	265	14	101.14
2	<i>C.steveii</i>	30	3.5	249.80
3	<i>C.triphyllum</i>	514	31.2	345.36
4	<i>C.ritchii</i>	760	67	32.23
5	<i>C.crocifolium</i>	122	11	369.56
6	<i>C.tauri</i>	195	15.5	84.90
7	<i>C.tunicatum</i>	73	5.8	23.60
8	<i>C.brachyphyllum</i>	145	15	44.13

Plant's Samples Preparation

A portion, 0.5 g of each plant extract from each plant material was accurately weighed and placed into 100 mL Erlenmeyer flasks. Petroleum ether (analytical grade, 10mL x 2) was added with frequent shaking for 1hr, followed each time by filtration. Solid residues were air dried at room temperature and then extracted with 10mL HPLC-grade dichloromethane with frequent shaking for 45 min.

Then, ammonium hydroxide (12.5%), 0.5 mL, was added, followed by vigorous shaking for 15 min, and the mixture was left for 30 minutes. Afterwards, plant residues were filtered and the filtrates were saved in the refrigaerated. Plant residues were washed twice with 10 mL HPLC-grade dichloromethane and then filtered. The collected filtrates and the plant residue washes were combined. Organic phase (dichloromethane) was evaporated to dryness under vacuum and then dissolved in 1mL of 70% ethanol (absolute grade).

The ethanolic solution was filtered by 0.45 mL Teflon filter, 0.5mL was transferred into 2mL amber HPLC vials, and then 1mL of 70% ethanol was added. A portion, 25 µL, was injected into the HPLC. Two extraction replicates were prepared for each plant sample.

Three stock solutions of colchicine standard 1000, 2000 and 2000 ppm were prepared at different times (0-11 min., 11-15 min., and 15-20 min, respectively) by accurately weighing 10, 50 and 50 mg of colchicines reference standard (Fluka Chemie, from *C. brachyphyllum*) into 10, 25 and 25mL volumetric flasks, respectively, then 5, 10 and 10mL of HPLC-grade methanol were added, respectively, and the mixture was shaken until all solids were dissolved then was diluted to volume with HPLC-grade methanol and mixed to ensure complete solubility.

Chromatographic conditions

The HPLC analysis was performed on a Lachrom-MERCK-HITACHI (Tokyo, Japan), equipped with quaternary gradient L-7150 pump, L-7455 Diode-Array Detector, L-7200 auto-sampler, and D-7000 Interface. The analytical HPLC column used was LiChroCART_125-4, Purospher_ STAR RP-18 endcapped (5 mm). Mobile phase was a gradient blend of acetonitrile and 3% acetic acid in water in the following manner:

Flow rate used was 1mL min⁻¹, detector set at 245nm and injection volume was 25 mL. (-)-Colchicine eluted at 13.5 min; the total run time was 30 min. All plant samples, calibration points and QC samples were injected

twice. Measuring peaks areas, three linear calibration curves were constructed with r^2 values of 0.9992, 0.9995 and 0.9996. Two quality control (QC) samples at 30 and 90 ppm, 75 and 200 ppm and 20 and 50 ppm were accurate within 0.77 and 0.42%, 3.36 and 0.63% and 12.23 and 4.5% (ppm, RSD%) for the 1st, 2nd and 3rd calibration curves, respectively, from actual concentration⁽¹⁶⁾.

Sample Analysis

Plant samples were analyzed by the above-described method. All plant samples were injected twice, and the results obtained are given in Table 1. The percent of colchicine in the samples was calculated against external colchicine standard using the following equation:

$$\%W = W \text{ colchicine} \frac{1}{4} C_x FV_x D_x 100\%/W$$

Where;

C: is the sample's colchicine concentration (mg/mL), extrapolated from the calibration curve's linear regression.

FV: is the final volume of the sample in mL.

D: is the dilution factor.

W: is the sample weight in mg⁽¹⁶⁾.

REFERENCES

- (1) Nordenstam B. In The Families and Genera of Vascular Plants, K. Kubitzki (Ed.), Springer-Verlag, Berlin, 1998; 3: 175-185.
- (2) Clapham A.R., Tutin T.G., Warburg E.F. Flora of the British Isles, Cambridge University Press 1962; 2nd Edition, 982.
- (3) Wendelbo P., Staurt D. Colchicum L. In: Agriculture and Agrarian Reform Republic of Iraq, Baghdad. Townsend C.C. and Guest, 1985; 44-53.
- (4) Oran S.A., D.M. Al-Eisawi. Checklist of medical plants in Jordan. *Dirasat Med. Biol. Sci.* (in Arabic) 1998; 25, 84-112.
- (5) Feinbrun-Dothan N. Flora Palestina, The Israel Academy of Sciences and Humanities, Jerusalem 1986; 26-31.
- (6) Alexander P., Brigitte N., Meinhart Z. Immunoassays for the quantitative determination of Colchicine. *Planta Med.* 1994; 60, 77-83.
- (7) Pelletier P.J., Caventou, J.B. *Ann. Chim. Phys.*, 1820; 14: 69-73.
- (8) Herbert R.B. *The Biosynthesis of Secondary Metabolites*, 2nd Edition, Chapman and Hall, London, New York 1988; 120-181.
- (9) Dollery C., Boobis A., Rawlings M., Thomas S., Wilkings M.. Volume 1. Therapeutic Drugs, *Churchill Livingstone* 1999; 320-322.

Results and Discussion

Eight *Colchicum* spp., grown wild in Jordan, were analyzed for their colchicines alkaloid content. *C.crocifolium* showed the highest colchicines content with 369.56 μ g/ml, meanwhile the lowest is recorded in *C.tunicatum* with 23.60. *C. steveii* and *C.triphyllum* were close to each other in colchicines content, 249.80 and 345.36, respectively. *C.ritchii* has low content of colchicine (32.23%) and *C.brachyphyllum* (44.13).

However, a good colchicines percentage were seen in *C. Hierosolymmitanum* and *C.tauri* 101.14 and 84.90, respectively, similar data were seen by utilization of various plant parts in few *Colchicum* spp.⁽¹⁶⁾. We can conclude that Jordanian *Colchicum* spp. Jordanian *Colchicum* corms could be a good commercial source of the alkaloid colchicines. Similar results were obtained from different parts of *Colchicum* spp.⁽¹⁶⁾.

ACKNOWLEDGMENTS

The author would like to acknowledge the faculty of Pharmacy, The University of Jordan, Prof. Kholood Khamees, Prof. Ahmad Kofahi and Prof. Firas Seof for their great help and support during my sabbatical year (2010/ 2011) at the Faculty of Pharmacy, JUST. Without their inspiration I will not be able to do this work.

- (10) O'Neil M.J., Smith, A., Heckelman, P.E. The Merck Index, 13th Edition, Merck Research Laboratories, Division of Merck and Co., Inc., White House Station, NJ 2001; 432.
- (11) Terkeltaub R.A. Gout. *N. Engl. J. Med.* 2003; 349: 1647-1655 .
- (12) Drenth, J.P. J.W. van der Meer. *N. Engl. J. Med.* 2001; 345: 1748-1757.
- (13) Sakane T., Takeno, M. Novel approaches to Behçet's disease. *Expert Opin. Investig. Drugs* 2000; 9: 1993-95.
- (14) Eigsti O.J., Dustin J.P. Colchicine in Agriculture, Medicine, Biology, and Chemistry, The Iowa State College Press, Ames 1955; 255-265.
- (15) Kneeder W.H. Phytochemical studies and cytotoxicity evaluations of *Colchicum tunicatum*. *J. Amer. Med. Assoc.* 1945; 129: 272-75.
- (16) Alali F., Tawaha K. and El-Elimat T., Determination of (-)-demecolcine and (-)-colchicine content in selected Jordanian species. *Pharmazie*, 2007; 62: 739-742.

تحديد نسبة الكولشيسين في عدة أنواع من الكولشيكوم التي تنمو في الأردن

وليد الترك¹ وسليمان العليمات^{2*}

¹ كلية الصيدلة، جامعة الشرق الأوسط، عمان، الأردن.

² كلية الصيدلة، الجامعة الأردنية، عمان، الأردن.

ملخص

تم جمع ثمانية أنواع من نبات الكولشيكوم، والذي ينمو برياً في الأردن، من عدة مناطق في المملكة. وتم تحديد المركب الرئيس، قلويد الكولشيسين، باستعمال جهاز كروماتوغرافيا السائل ذو الضغط العالي. وقد أثبتت نتائج البحث أن نوع كولشيكوم كروسيفوليوم يحتوي على أعلى نسبة من الكولشيسين.

الكلمات الدالة: أنواع الكولشيكوم، كولشيسين، نباتات، الأردن.

تاريخ استلام البحث 2015/1/25 وتاريخ قبوله للنشر 2015/4/20.