

## LC-MS Analysis of Corms of *Colchicum hierosolymitanum* (Colchicaceae)

Feras Alali<sup>1\*</sup>, Abdullah El-Alali<sup>2</sup>

<sup>1</sup> Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan

<sup>2</sup> Department of Chemistry, Mu'tah University, Alkarak - Jordan.

---

### Abstract

LC-MS analysis of corms of *Colchicum hierosolymitanum* Feinbr (Colchicaceae) was conducted using a single quadrupole mass analyzer equipped with (+)-APCI ionizing interface. (-)-Colchicine (7) and eight (1-6, 8, 9) natural analogues were detected and identified in the alkaloid rich fraction. These compounds are reported for the first time in *C. hierosolymitanum*.

**Key words:** (-)-Colchicine; *C. hierosolymitanum*; (+)-APCI LC-MS; Jordan

---

### Introduction

The genus *Colchicum* belongs to the family Colchicaceae (formerly Liliaceae), comprising a worldwide of 100 species (Wendelbo and Stuart, 1985). Nine *Colchicum* species grow in Jordan: *Colchicum brachyphyllum* Boiss. & Hausskn., *Colchicum crocifolium* Boiss., *Colchicum hierosolymitanum* Feinbr, *Colchicum tauri* Siehe ex Stefanov, *Colchicum ritcii* R. Br., *Colchicum shemperi* Janka & Stefanov, *Colchicum steveni* Kunth, *Colchicum triphyllum* G. Kunze, and *Colchicum tunicatum* Feinbr. (Al-Eisawi, 1998). The medicinal value of the genus *Colchicum* is attributed to the presence of colchicinoids, mainly (-)-colchicine. Today (-)-colchicine is still the drug of choice to relieve gout acute attacks. (-)-Colchicine and its analogues are used clinically for the treatment of certain forms of leukemia and solid tumors. Due to its potent affinity for tubulin, (-)-colchicine is also used in biological and breeding studies to produce polyploidy, multiplication of the chromosomes in cell nucleus, and in tubulin binding assays as a positive control (Trease and Evans, 1983; Poutaraud and Champay, 1995).

*Colchicum hierosolymitanum* Feinbr (Colchicaceae), meadow saffron, is a perennial herb with big underground corms covered by onion-like scales. Flowering time is between October-November (Al-Eisawi, 1998). Due to the fact that the corms are poisonous, local people in Jordan call it "abokhunag" which means the plant that causes suffocation.

---

\* Corresponding author: e mail: [falali@just.edu.jo](mailto:falali@just.edu.jo)

Liquid chromatography-mass spectroscopy (LC-MS) analysis of plant extracts is becoming a very valuable tool to detect the presence of certain constituents before aiming a large-scale purification and to minimize dereplicative phytochemical studies (Gu *et al.*, 1997; He *et al.*, 1997). Two ionizing interfaces are widely used to couple with liquid chromatography (LC), atmospheric pressure electrospray ionization (APESI) and atmospheric pressure chemical ionization (APCI) (Whitehouse *et al.*, 1985). These relatively new ionization interfaces provide “soft” ionization in comparison with the widely used electron impact (EI) and chemical ionization (CI) (Whitehouse *et al.*, 1985). Thus, fragmentation occurs in ESI and APCI to a much lesser extent than to those of in EI or CI. Fragmentation mechanisms in APESI and APCI are not well studied. Detection of positive and negative parent ions is quite feasible using either full scan (FS) mode or selected ion monitoring (SIM) mode. By utilizing ion extraction from a full scan acquisition, i.e. total ion current (TIC), one could search for all expected masses; molecular ions and fragments of plant constituents. While thin layer chromatography (TLC) is widely applied to detect known compounds in a new species, TLC possesses low resolution and several isomers could elute in a single spot. LC/MS provides efficient, sensitive, high resolution, and unequivocal tool to judge the presence of known and new compounds in new species. This will cut both time and money spent on tedious extraction, fractionation, and phytochemical analysis of the new species. Thus, qualitative LC-MS analysis of plant extracts should be encouraged to explore the chemical diversity early in the process of plant’s phytochemical analysis.

LC-MS analysis of (-)-colchicine and analogues in animals serum and milk, and human plasma were recently reported (Sannhoe *et al.*, 2002; Sutherland *et al.*, 2002; Hamscher *et al.*, 2005). This paper describes the first direct analysis of colchicinoids in a plant extract.

## Materials and Methods

*Plant Collection and Extraction:* Whole plant, 1 kg, was collected in November, 2000 from Erhaba, northern Jordan. The collected plants were identified by Prof. A. El-Oklah, a plant taxonomist, Department of Biological Sciences, Faculty of Science, Yarmouk University, Irbid, Jordan and a voucher specimen (No. 200) was kept at the medicinal plant museum of the Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan. The corms were separated, sliced, and left to dry. The dried materials, 0.16 kg, were soaked in methanol for two days and this step was repeated three times to ensure complete recovery. The methanolic residue was brought to dryness, yielded 26.9 g, by a Rotavap. The crude alkaloid isolation was carried out according to the scheme of Santavy *et al.* (1982). Methanolic residue was dissolved in 5 % acetic acid and extracted three times with petroleum ether (fraction A) in order to remove non-alkaloid substances. The aqueous acid residue was then extracted three times with ethyl ether (fraction B). The residue was then made alkaline, pH 9, with ammonium hydroxide and then extracted three times with chloroform (fraction C). Chloroform extract was evaporated to dryness and yielded 0.67 g. Two mg sample of standard (-)-colchicine (purchased from Sigma®) and fraction C were dissolved in 10 mL HPLC mobile phase and then transferred to 2 mL HPLC vials for LC/MS analysis (see below).

### *Liquid Chromatography-Mass Spectroscopy*

*Analysis:* Single quadrupole aQa Finnigan® LC/MS coupled with APCI interface and TSQ LC gradient pump was used for the analysis. The following chromatographic conditions were

applied: gradient system starting with 90% aqueous acetic acid (3% acetic acid in water):10% acetonitrile and ends with 60% aqueous acetic acid and 40% acetonitrile in 15 min, flow rate was 1 mL/min, HPLC column used was Lichro CART (125 × 4 mm) C<sub>18e</sub> RP (5 μm), injection volume was 50 μL. The mass detector conditions were as follows: APCI positive ionization mode, full scan mode from 150 to 550 amu, aQa source voltage set at 40 v, minimum scan per peak was 10, probe voltage was 3 kv, and probe temperature set at 400 °C.

## Results and Discussion

While chemical structures can not be determined with absolute certainty using LC-MS alone, the information obtained from the APCI mass analysis and TIC are still very valuable and very much informative. Among the objective of this study is to prove the power of this relatively new technique in predicting possible constituents. For example, if you find that a particular compound is 14 mass units less than a parent compound, this most likely means either a methoxy group is replaced by a hydroxyl or one less methylene group. Here we have to rely on fragmentation pattern, elution time, solid background in natural products biosynthesis and authentic standard, if available, to confirm the chemical structure.

Colchicum species: *Colchicum brachyphyllum* Boiss. & Hausskn, *Colchicum tauri* Siehe ex Stefanov and *Colchicum tunicatum* Feinbr Structural elucidation of the compounds detected in this species was based on LC-MS analysis and confirmed by comparing their retention times and mass spectra to those of authentic samples. All compounds identified in this species were isolated in current phytochemical works in our laboratories. (manuscripts are under preparation). These authentic compounds were identified by 1D- and 2D-nuclear magnetic resonance (NMR) and (+)-APCI mass analysis in collaboration with Research Triangle Institute, North Carolina, USA (spectra are available upon requests).

The APCI mass spectrum of (-)-colchicine (7) displayed the following major peaks  $m/z$ : 399.8 and 400.7 (molecular ion and base peak), 381.7 (-H<sub>2</sub>O), 357.7 (-COCH<sub>3</sub>), 340.7 (-H<sup>+</sup>, -NHCOCH<sub>3</sub>), 326.7, 309.7, and 281.6. (-)-Colchicine was the major peak, 9.39 min, in fraction C (Figure 1). This was confirmed by comparison with APCI mass spectrum and HPLC retention time of standard (-)-colchicine.

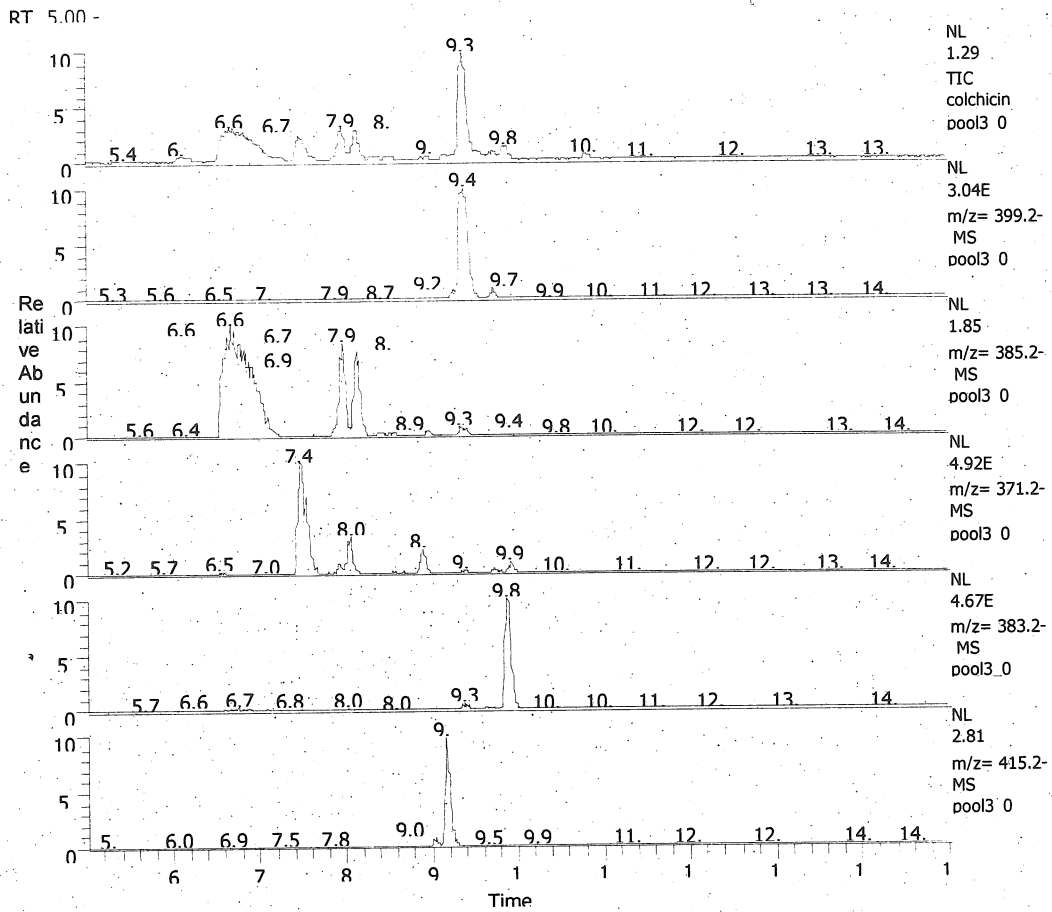


Figure 1. (+)-APCI LC/MS analysis of corms<sup>2</sup> extract of *Colchicum hierosolymitanum*.

Retention time (min)	Compound	APCI main mass peaks ( $m/z$ ) (%intensity)*
3.51	(-)-3-Demethylcolchicine (1)	371.7(100), 340.7(16.7), 278.6(5.6)
6.12	(unknown)**	326.7(100), 319.7(9.3), 298.7(13.9), 278.6(47.2), 266.6(33.3), 238.6(13.9)
6.69	(-)-Colchicine (2)	385.7(100), 354.7(19.4), 342.7(2.8), 323.7(2.8), 278.6(2.8)
7.49	(-)-Demecolcine (3)	371.7(100), 356.7(9.2), 341.7(13), 340.7(24), 339.7(26), 325.6(13.9), 309.6(27.8), 278.6(18.5)
7.98	(-)-3-Demethylcolchicine (4)	385.7(100), 367.7(5.6), 343.7(13), 326.7(5.6), 311.6(3.7), 266.6(3.7)
8.15	(-)-2-Demethylcolchicine (5)	385.7(100), 367.7(5.6), 343.7(15.7), 326.7(7.4), 311.6(6.5), 266.6(5.6)
9.16	(-)-Colchifoline or (-)-Colchicine (6) **	415.7(100), 341.7(6.5), 326.7(20.4), 278.6(23.1)
9.37	(-)-Colchicine (7)	399.8(100), 381.7(15.4), 357.7(20), 340.7 (15.4), 326.7(10.7), 281.6(7.8)
9.76	$\beta$ -Lumicolchicine (8)	399.8(100), 381.7(21.3), 342.7(29.7), 326.7(36.1), 278.6(14.8)
9.89	(-)-Cornigerine (9)	383.7(100), 369.7(14.8), 341.7(16.7), 326.7(13), 309.8(5.6), 296.8(7.4), 266.6(9.2)
10.82	(unknown) **	340.7(100), 319.7(11.1), 312.7(6.5), 281.6(17.6), 278.6(33.3)

Table 1. Characteristic (+)-APCI mass peaks of *Colchicum hierosolymitanum* constituents.

\* Molecular ion is the base peak for all compounds

\*\* Preparative isolation and NMR analysis needed to prove the structure.

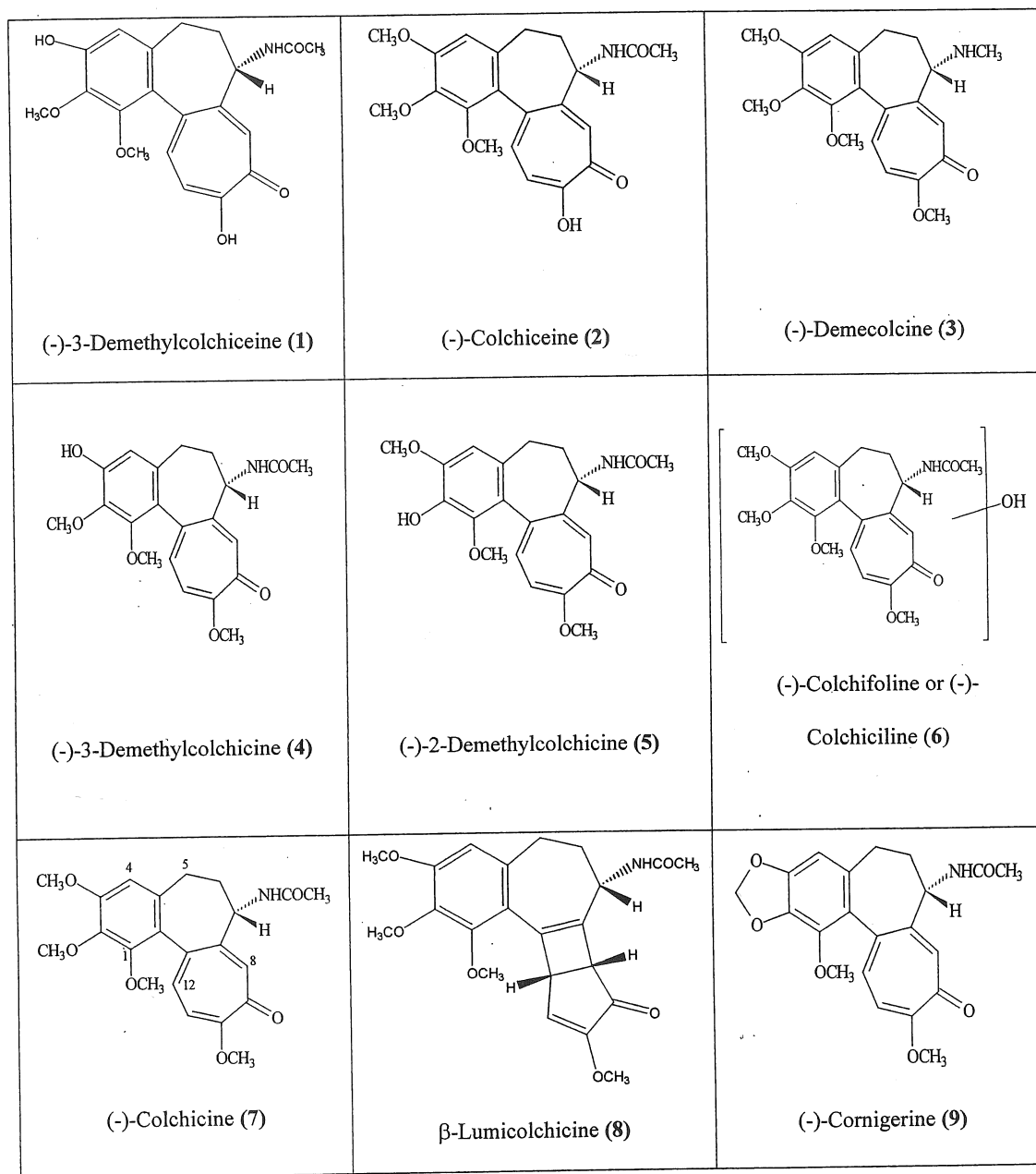


Figure 2. Colchicine-like compounds in the alkaloid rich fraction of corms of *Colchicum hierosolymitanum* by (+)-APCI LC/MS analysis.

Eight colchicine-like compounds were detected in the chromatogram (1-6, 8, 9) (Figure 2). Using mass extraction of the full scan (TIC) chromatogram, the following molecular ions  $m/z$  were observed: 399.8 (9.37 and 9.76 min), 385.7 (7.98, 8.10, and 6.69 min), 371.7 (3.51 and 7.49 min), 383.7 (9.89 min), 340.7 (10.82), 326.7 (6.12), and 415.7 (9.10 min) (Figures 1 and 2, Table 1). (7) at positions C-1, 2, 3, and 10. This compound is confirmed to be (-)-3-demethylcolchicine (1) by comparing its mass spectra and retention time with an authentic sample. (-)-Cornigerine (9), a molecular ion peak at  $m/z$  383.7, is easily identified showing similar patterns of fragmentation to that of (-)-colchicine but with all characteristic mass peaks differed by 16 amu due to the presence of the methylene-dioxy ring. This compound was also The presence of two compounds having  $m/z$  399.7 as molecular ion with different fragmentation patterns and at close retention times indicate the presence of (-)-colchicine (7) at 9.37 min and its photoisomer  $\beta$ -lumicolchicine (8) at 9.74 min. This was confirmed by comparing  $\beta$ -lumicolchicine mass spectra and retention time with an authentic sample. Compounds with molecular ions of  $m/z$  385.7, 14 amu less than that of (-)-colchicine, have similar chemical structure to (-)-colchicine, but with a hydroxyl group instead of one methoxy. Identified compounds were (-)-colchicine ((-)-10-demethylcolchicine) (2), (-)-3-demethylcolchicine (4), and (-)-2-demethylcolchicine (5). Again, this was confirmed by comparing their mass spectra and retention times with authentic samples. Peaks at 7.49 min and 3.51 min both showed a molecular ion of 371.7  $m/z$ . Regarding the compound that elutes at 7.49 min, the mass spectrum indicate an amino-acetyl group in (-)-colchicine is now replaced by a amino-methyl (3). This compound, at 7.49 min, is (-)-demecolcine (3). This was suggested by molecular ion fragmentation to at  $m/z$  356.7 (-CH<sub>3</sub>) and at  $m/z$  340.7 (-H<sup>+</sup>, -NHCH<sub>3</sub>). This compound showed identical mass fragmentation patterns and HPLC retention time with to those of an authentic sample. Judging from molecular ion, fragmentation pattern and early elution, the compound at 3.51 min (1) was expected to be similar to (-)-colchicine (7) but with two methoxy groups and two hydroxyl groups instead of four methoxy groups in (-)-colchicine reported to be present in other Jordanian *Colchicum* species (Tal, 1990). This was also confirmed by comparing its mass spectra and HPLC retention time with those of the authentic sample. We could not prove the structure of the two compounds eluted at 6.12 and 10.82 min nor confirm the hydroxylation site in compound (6).

The method used in this study, LC-MS-(+)-APCI, has the advantage of using a soft ionization over the harsh classic ionization techniques like EI and CI. It is expected that fragments will be more intense in EI or CI but their molecular ion will be much weaker. While FAB is also considered a soft ionization technique, it is not yet developed to be coupled on-line with HPLC. Also, this acidic-based HPLC method significantly improves both the positive protonation and ionization of colchicinoids and their chromatographic peak shapes.

LC-MS analysis of plant extract is a powerful technique to detect the presence of known compounds and predict new compounds before aiming a large-scale phytochemical work. We predict that this technique will be applied more and more to minimize dereplicative phytochemical works on species of the same genus. In this study we are able to detect and identified (-)-colchicine and the related eight analogs by a simple one analytical injection of an alkaloid rich fraction.

## Acknowledgement

The authors would like to thank Prof. Naji Najib, director of International Pharmaceutical Research Center, Amman, Jordan for kindly giving an opportunity to access on the LC/MS

instrument. Thanks are also due to Mr Ez Ghanem and Mrs Ruba Al-khartabeel for the technical help.

## References

Al-Eisawi D. (1998). Wild Flora of Jordan and Neighboring Countries. Amman, Jordan. Commercial Press, Jordan Press Foundation "ALRAI".

Gu Z.-M., Zhou D., Lewis N., Wu J., Shi G., and McLaughlin J.L. (1997). Isolation of new bioactive annonaceous acetogenins from *Rollinia mucosa* guided by liquid chromatography/mass spectrometry. *Bioorg. Med. Chem.*, 5: 1911-1916.

Hamscher G., Priess B., Nau H., and Panariti E. (2005). Determination of colchicines residues in sheep serum and milk using high-performance liquid chromatography combined with electrospray ionization ion trap mass spectrometry. *Anal. Chem.* 77: 2421-2425.

He X., Lin L., and Lian L. (1997). Electrospray high performance liquid chromatography-mass spectrometry in phytochemical analysis of Kava (*Piper methysticum*) extract. *Planta Med.*, 63: 70-74.

Poutaraud A., and Champay N. (1995). Meadow saffron (*Colchicum autumnale* L) a medical plant to domesticate. *Revue Suisse – d'Agriculture.* 27 : 93-100.

Sannohe, S., Makino, Y., Kita T., Kuroda, N., and Shinozuka T. (2002). Colchicine poisoning resulting from accidental ingestion of meadow saffron (*Colchicum autumnale*). *J. Forensic. Sci.* 47: 1391-1396.

Santavy F., Dvorackova S., Simanek V., and Potesilova H. (1982). Isolation and identification of alkaloids of the subfamily Wurmbaeioideae. *Medical Faculty, Palacky University, Olomoc, Czech Republic. Fac. Med.* 105-163.

Sutherland F.C., Smit M.J., Herbst, L., Els J., Hundt H.K., Swart K.J., and Hundt A.F. (2002). Highly specific and sensitive liquid chromatography-tandem mass spectrometry method for the determination of 3-desmethylthiocolchicine in human plasma as analyte for the assessment of bioequivalence after oral administration of thiocolchicoside. *J. Chromatogr. A.* 949: 71-77.

Tal T. H. (1990). Msc Thesis, Faculty of Science, University of Jordan, Amman, Jordan.

Trease S. E., and Evans D. (1983). Pharmacognosy, *Colchicum* seed and corm. 12<sup>th</sup> edition, pp. 593 - 597.

Wendelbo P., and Stuart D. (1985). *Colchicum* L., In: *Agriculture and Agrarian Reform, Republic of Iraq, Baghdad*, C. C. Townsend and Guest, pp. 44-53.

Whitehouse C.M., Dreyer R.N., Yamashita M., and Fenn J.B. (1985). Electrospray interface for liquid chromatographs and mass spectrometers. *Anal. Chem.*, 57: 675-679.

Received: 11.12.2004

Accepted: 01.03.2005