Journal of Medicinal Plants Research Vol. 5(29), pp. 6515-6522, 9 December, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals
DOI: 10.5897/JMPR11.482

Full Length Research Paper

Tropane alkaloids of *Atropa belladonna* L. and *Atropa acuminata* Royle ex Miers plants

Fatemeh Ashtiania and Fatemeh Sefidkonb

[1]Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran.
[2]Research Institute of Forests and Rangelands, P.O. Box: 13185-116, Tehran, Iran.

Accepted 7 October, 2011

The purpose of the present study was to determine the tropane alkaloid content in different parts of the wild and cultivated *Atropa belladonna* L. and wild *Atropa acuminata* Royle ex Miers in Iran. Determination of alkaloids was performed by high-performance liquid chromatography (HPLC) method. Samples were extracted with chloroform- methanol- cc. ammonia 15:15:1(*v*/*v*/*v*). HPLC separation was performed on two C_8 columns. An isocratic mobile phase of acetonitrile- 50 mM phosphate buffer (pH 2.95) 10:90 and 20:80(v/v) was used. Peaks were identified by standards and diode-array detection. Scopolamine and atropine were determined by external method at 210 nm. The total alkaloid content of the *Atropa belladonna* L. of sari-kiassar road was determined by a HPLC method to be 2.88% for the leaves and 8.06% for the roots and 1.42% for the stem. The total alkaloid content of the cultivated *Atropa belladonna* L. was 1.76% for the leaves, 3.3% for the roots, 1.42% for the stem and 4.82% for the seeds. Total alkaloid content of the leaves of *A. acuminata* was 5.88%. In all samples the percentage of atropine was higher than scopolamine. The results showed that *A. belladonna* and *A. acuminata* constitutes the major storage site and had the highest amount of these alkaloids. These alkaloids may be transported to other plant organs providing potential deterrent and antimicrobial activities.

Key words: *Atropa belladonna* L., *Atropa acuminata* Royle ex Miers, high-performance liquid chromatography (HPLC), Tropane alkaloids.

INTRODUCTION

Atropine, hyoscyamine and hyoscine (or compounds, which render extraction and purification scopolamine) are alkaloids obtained from solanaceae plants (Reynolds and Martindale, 1993). They are also found in the plant families for example, Erythroxylaceae, Convolvulaceae, Proteaceae, Orchidaceae, Euphorbiaceae, Cruciferae, Rhizophoraceae (Evans, 1979) and in the fungus *Amanita muscaria* (Willaman and Li, 1970). Their common structural element is the azabicyclo [3.2.1] octane system, and over 150 tropane alkaloids have been isolated.

Atropa is a perennial plant, native to central, Southern Europe and cultivated worldwide. It contains tropane alkaloids, which possess anticholinergic and spasmolytic properties (Tyler et al., 1988); they are commonly used as an anaesthetic and spasmolytic and in eye surgery. These are a series of secondary metabolites that have been mainly described in the Solanaceae family and initially in the genus Atropa that act as feeding deterrents and antimicrobial defenses for the plants (Waterman, 1993). *A. acuminata* is also a perennial plant that grows to 0.9 m by 0.75 m. It is in flower from June to August, and the seeds ripen from August to October. The flowers are hermaphrodite (have both male and female organs) and are pollinated by Insects. Analytical methodologies are available for the quantitative determination of the alkaloids including spectrophotometry (El-Masry, 1973; British pharmacopia, 1993b; US pharm., 1995; Hassan and Davidson, 1984), fluorometry (Gfeller, 1979 et al), high-performance liquid chromatography (Gfeller, et al 1979; Pennington, 1982; Lund and Hansen, 1978), gas-liquid chromatography (British pharmacopia, 1993b;

*Corresponding author. E-mail: Sefidkon@rifr-ac.ir.

Abbreviation: HPLC, High-performance liquid chromatography.

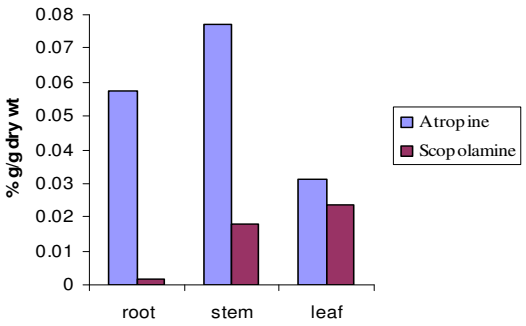

Figure 1. Amount of tropane alkaloids in different tissue of the native *A. belladonna*.

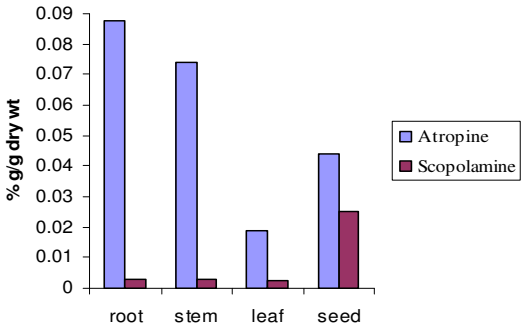

Figure 2. Amount of tropane alkaloids in different tissue of the cultivated *A. belladonna*.

US pharm., 1995; Majlat, 1982; Ballbach, 1977) and potentiometric titration (British pharmacopia, 1993a).

In this paper, we reported the determination and quantification of tropane alkaloids from different mature tissues of *A. belladonna* and *A. acuminata*, by high-performance liquid chromatography (HPLC) and compare the contents of atropine and scopolamine in different parts of cultivated and wild *Atropa belladonna* in Iran and also *Atropa acuminata*.

MATERIALS AND METHODS

Plant material

A. belladonna were collected from the sari-kiassar road and Gilan: asalem-khalkhal (Iran) in May, 2006; and 1977 (Herbarium sample) in the full flowering stage. *A. acuminata* was collected from the Gorgane: gonbad-gholidagh (Iran) in June, 1986 (Herbarium sample) in the full flowering stage.*A. belladonna* were cultivated in Medicinal Plants and Drugs Research Institute and collected in September, 2006 when the fruit are matured and seed dispersal had occurred.

Chemicals

Scopolamine, atropine, acetonitrile and methanol of HPLC grade were purchased from Sigma-Aldrich, water (HPLC grade).

Alkaloid extraction

The powdered materials (0.5 g) obtained from Atropa species was extracted three times for 30 min in 15 ml of chloroform, methanol and 25% ammonia 15:15:1(*v*/*v*/*v*) using an ultrasound device. Then kept at room temperature for 1 h and filter through paper filter and washed twice with 1 ml $CHCl_3$, the solvent evaporate to dryness.

The residue was dissolved in 5 ml $CHCl_3$ and 2 ml of 1 N sulfuric acid and mix thoroughly. $CHCl_3$ fraction removed and the aqueous solution basified (pH 10) with 25% ammonium hydroxide on ice. Alkaloids were extracted once with 2 ml $CHCl_3$ and twice with 1 ml chloroform.

After the addition of anhydrous Na_2SO_4, it is filtered and residue washed with 1-2 ml $CHCl_3$. The solvent was evaporated to dryness under vacuum at 40°C and residue (total extract) was dissolved in appropriate volume (1-2 ml) of methanol (Kamada et al., 1986).

Reference alkaloids

The standard solution of Scopolamine and Atropine (0.01 g) in 100 ml methanol was prepared.

High performance liquid chromatography (HPLC)

Quantitative determination of the main tropane alkaloids, atropine and scopolamine was performed by HPLC employing a Knauer and Teknokroma system equipped with a K-1001 pump and a manual injector. The UV detector was a 210 λ_{max} and the column used was a packed with 25 × 0.46 cm Eurospher-100C_8 (knauer, Germany, A) and Lichrospher 100 RP_8 (Teknokroma, Spain, B), packed 5 µm particles (Figures 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12).

The isocratic mobile phase was a mixture of 10 and 20% acetonitrile and a buffer containing 50 mM sodium dihydrogen orthophosphoric acid, adjusted to pH 2.95 with orthophosphoric acid for A and B columns. Sample injection was 20 µl, and the analysis was performed at a flow rate of 0.8 and 1.0 ml/min for the 10 min, detection was conducted at 210 nm. The data were generated using a ChromeGate, employing atropine and (-) scopolamine as standard samples.

Quantification of the alkaloids

Quantitative determination was performed by external standard method. The standard solutions containing atropine and scopolamine (4, 10, 25, 50, 100, 200, 400 ppm) were prepared in methanol. A 20 µL volume of each standard solution was injected onto the HPLC column. The calibration graphs for atropine and scopolamine were constructed by plotting the peak area of the alkaloids versus their construction (Table1).

RESULTS AND DISCUSSION

Our data indicate that the distribution of alkaloids in *A. belladonna* seem to be tissue-specific. The results of the quantitative analysis of the main tropane alkaloids (atropine and scopolamine) from *A. belladonna* plant tissue showed a significantly higher amount of atropine than scopolamine in all samples studied (Figures 1 and 2). Results, although from different extraction and analytical methods, have reported previously (Harborne et al., 1993), showing higher amounts of atropine than

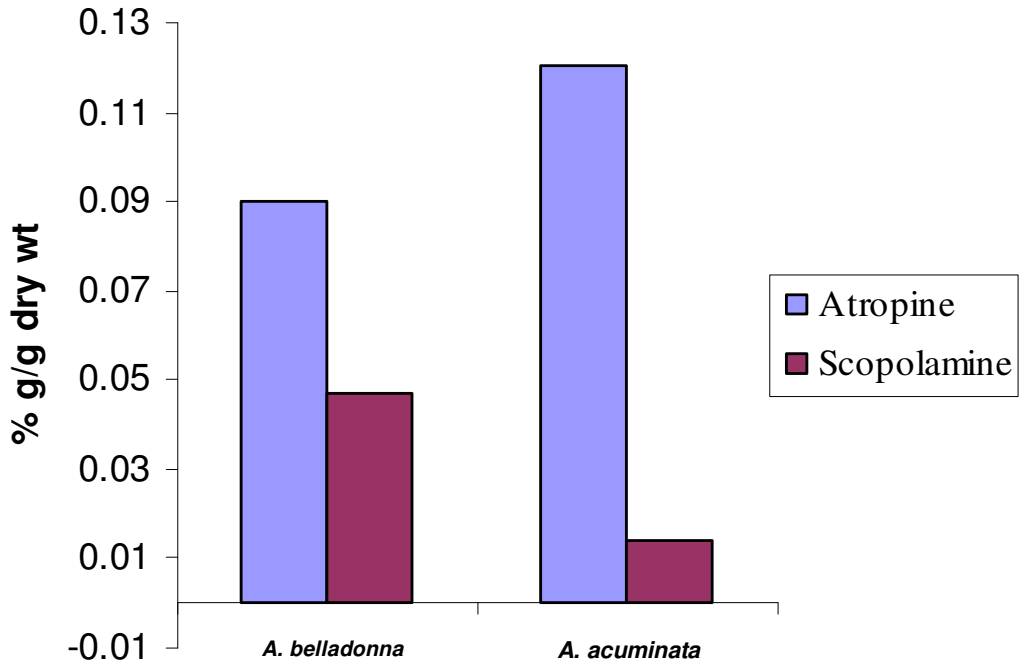

Figure 3. Amount of tropane alkaloids in leaves of the herbarium sample of Atropa plants.

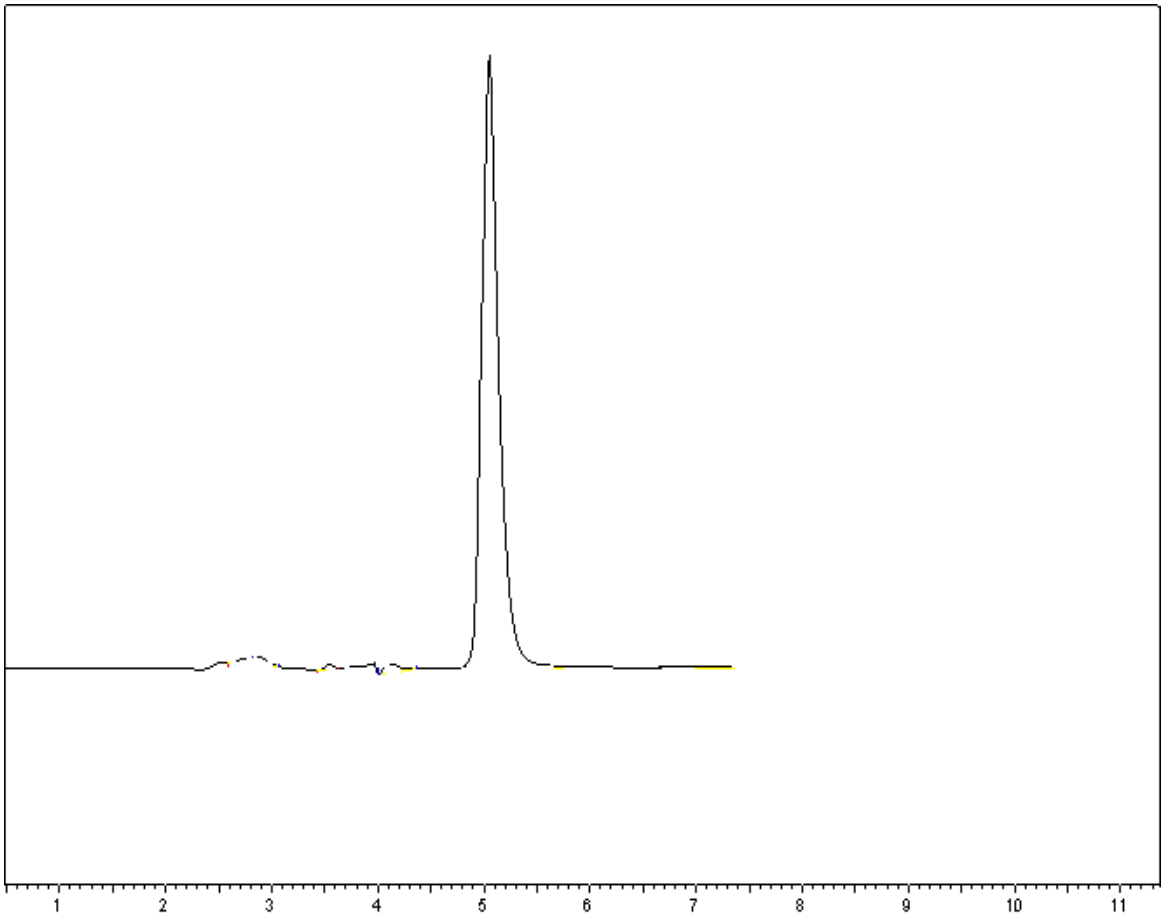

Figure 4. Chromatogram of atropine standard.

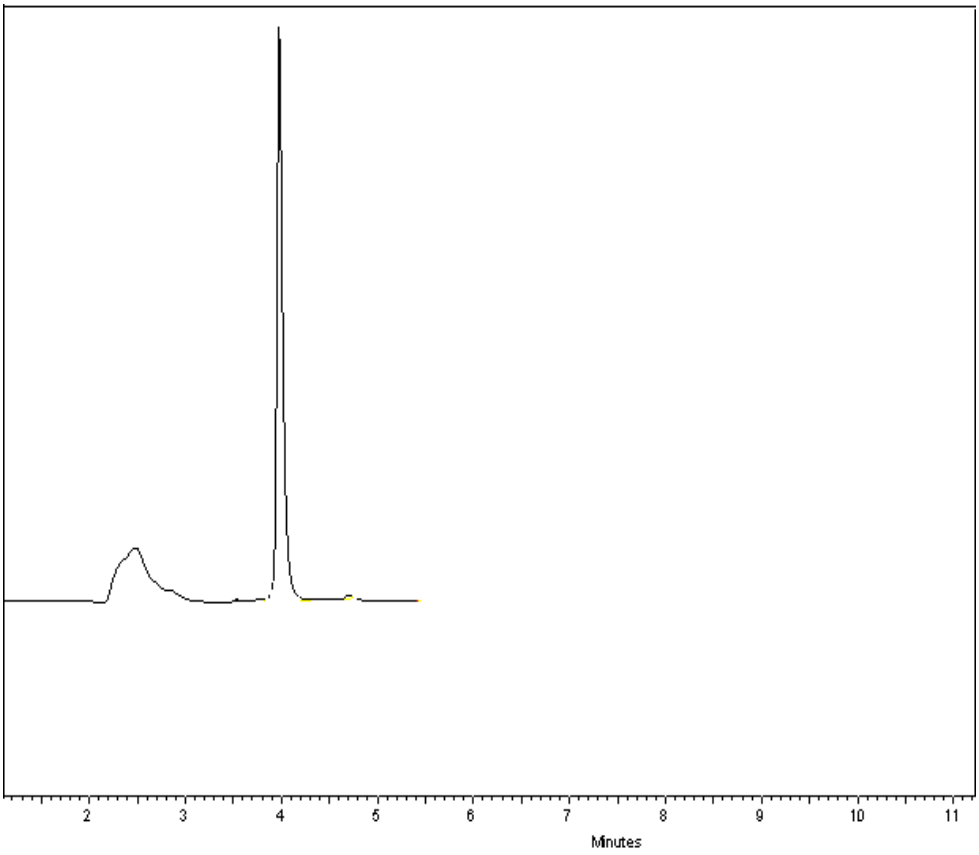

Figure 5. Chromatogram of scopolamine standard.

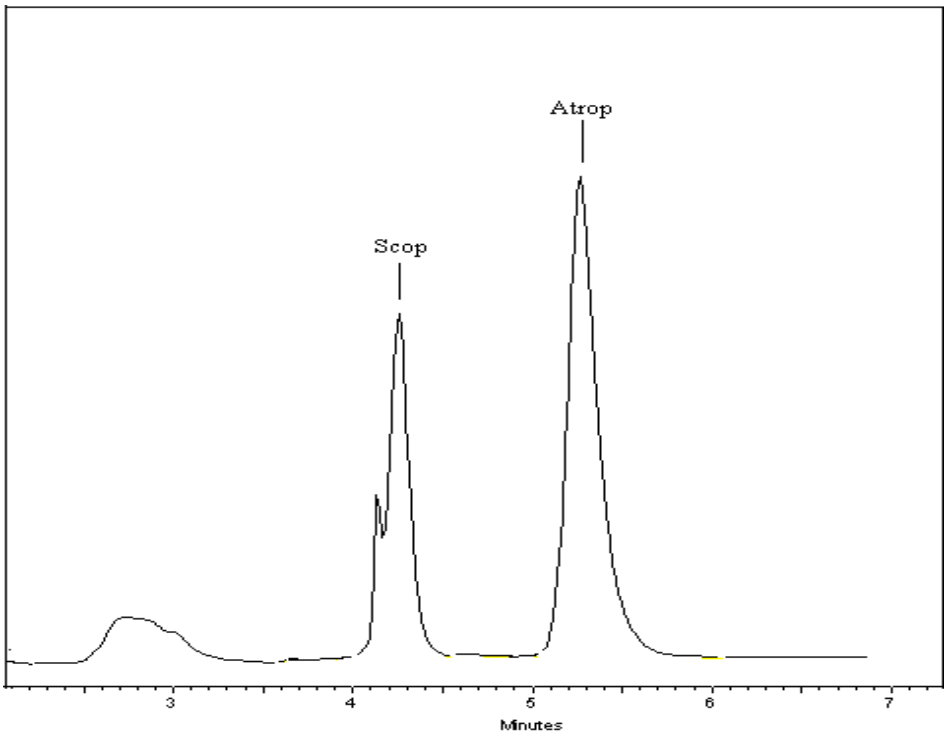

Figure 6. HPLC chromatogram of native Atropa belladonna leaf.

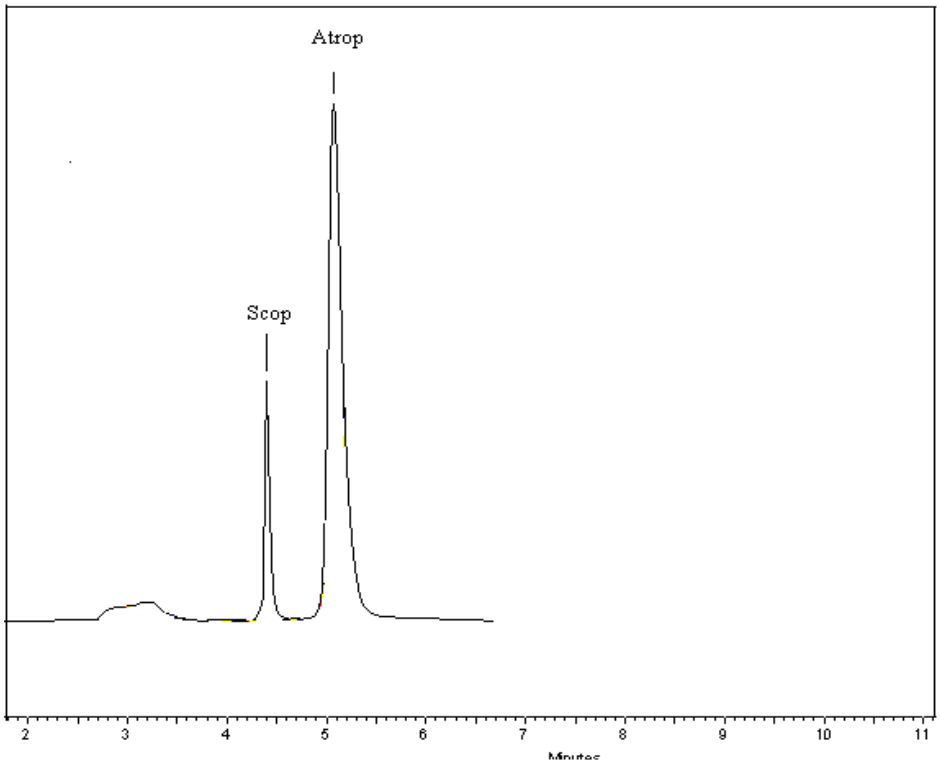

Figure 7. HPLC chromatogram of native Atropa belladonna stem.

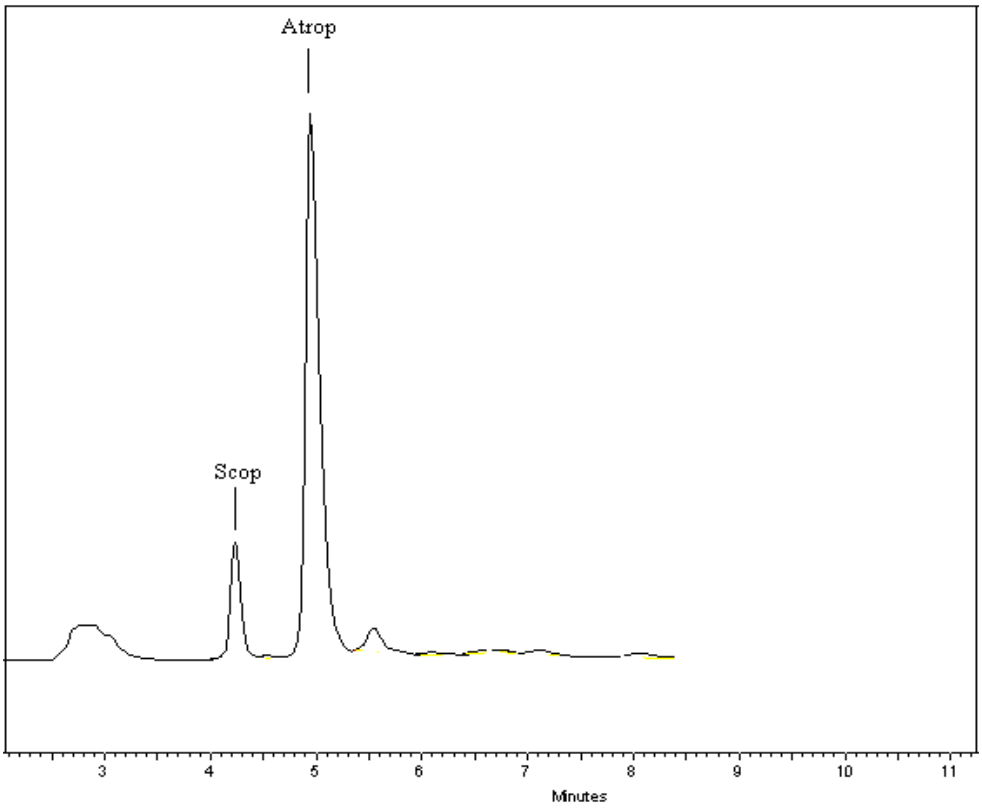

Figure 8. HPLC chromatogram of cultivated Atropa belladonna seed.

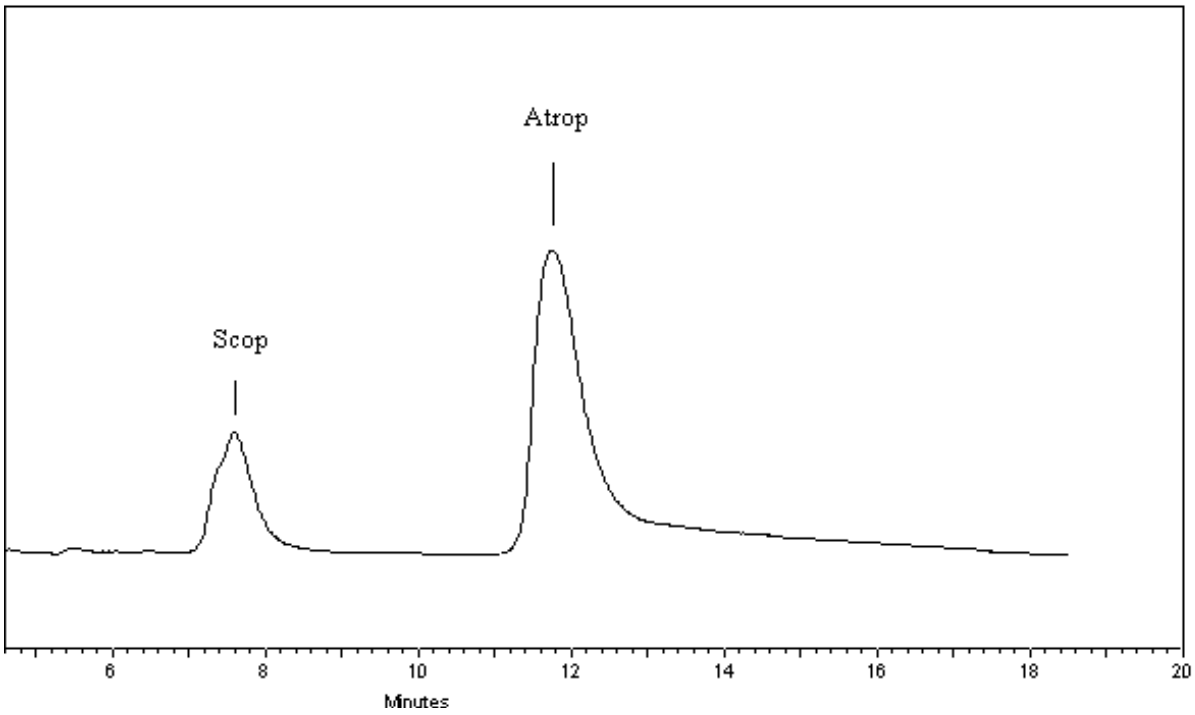

Figure 9. HPLC chromatogram of cultivated *A. belladonna* leaf.

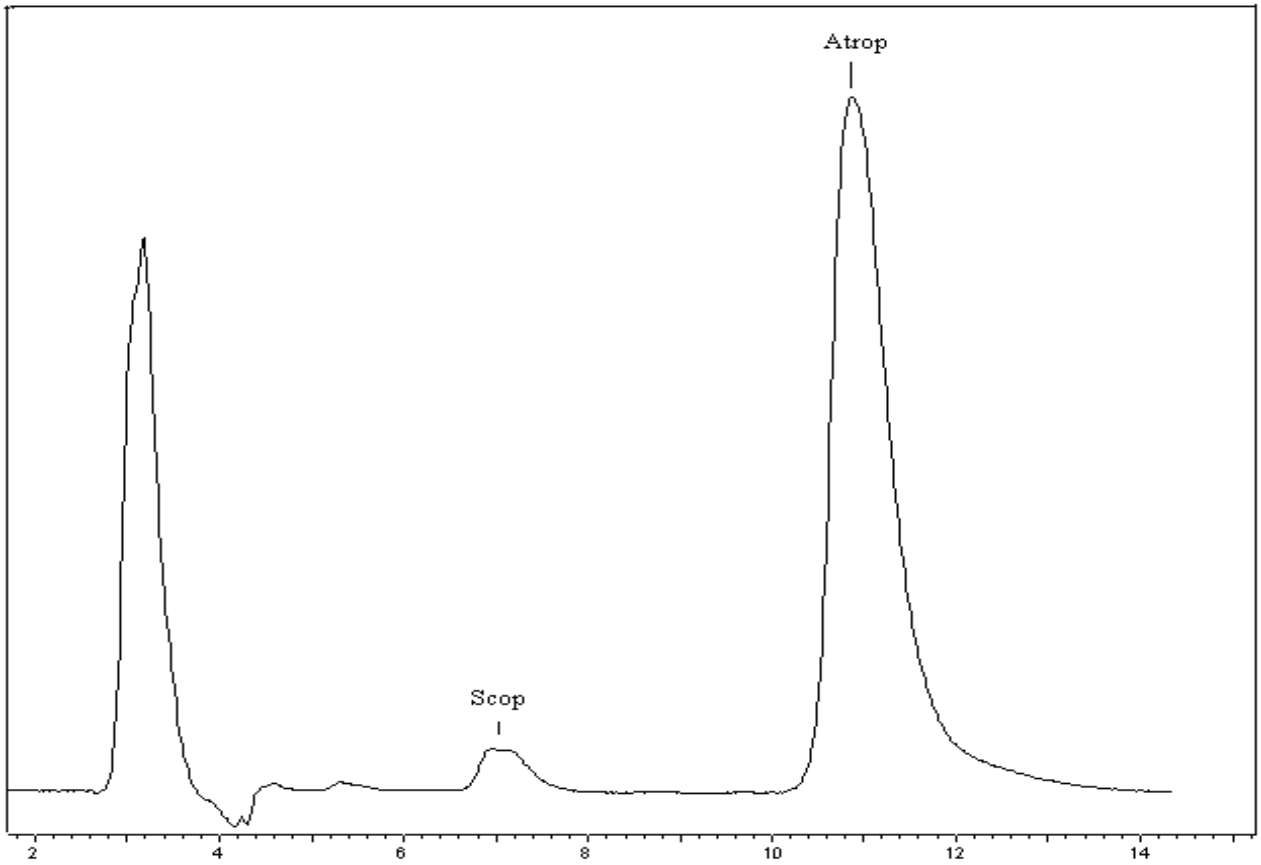

Figure 10. HPLC chromatogram of cultivated *A. belladonna* root.

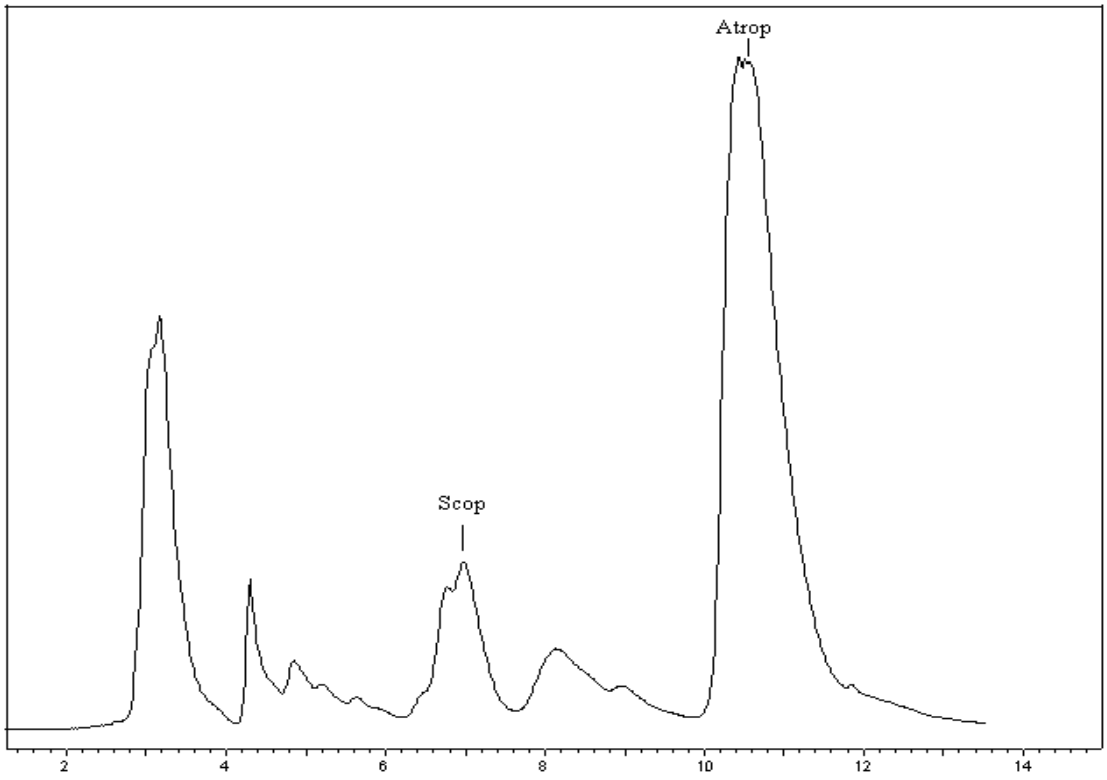

Figure 11. HPLC chromatogram of native *A. belladonna* root.

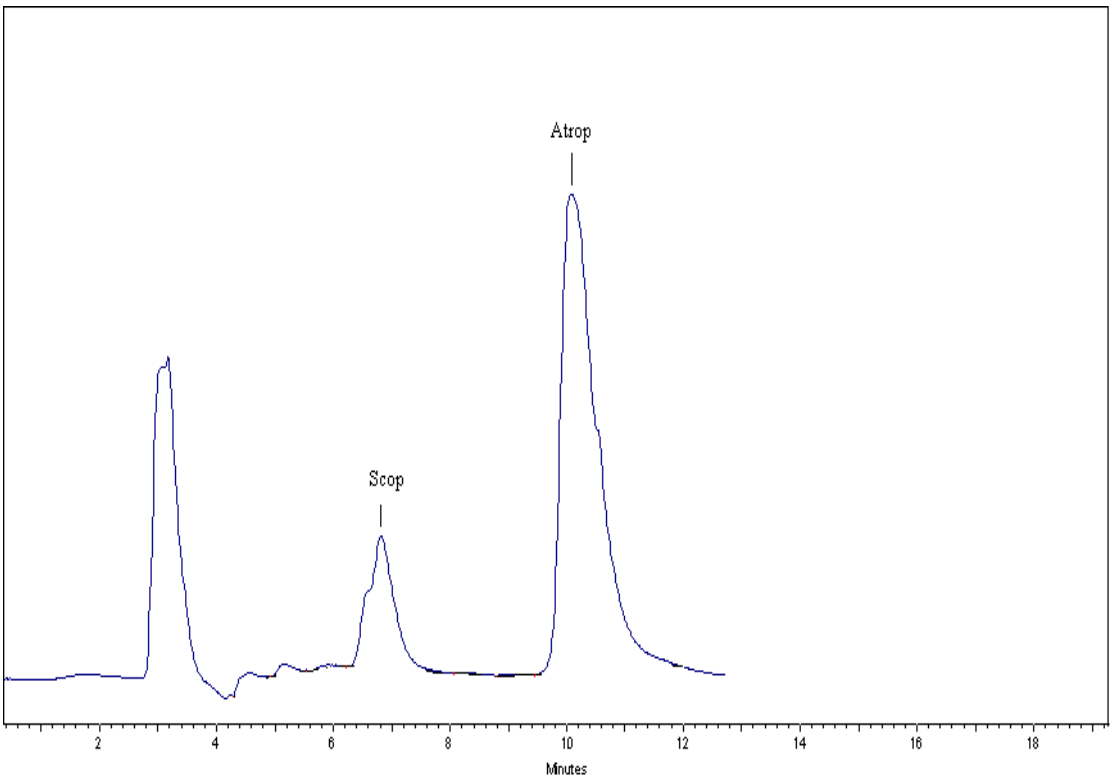

Figure 12. HPLC chromatogram of cultivated *A. belladonna* stem.

Table 1. Amount of total extracts (%) determined in different tissues of Atropa plants.

Sample		Stem	Root	Leaf	Seed
A. Belladonna L.	Native	2.55	8.06	2.88	-
	Cultivated	1.42	3.3	1.76	4.82
	Herbarium	-	-	5.7	-
A. acuminata Royle ex Miers	Herbarium	-	-	5.88	-

Table 2. Amount of tropane alkaloids (atropine and scopolamine) determined in different tissues of Atropa plants (% dry weight).

Compound	*A. belladonna*								*A. acuminata*
	Native			Cultivated				Herbarium	Herbarium
	Root	Stem	Leaf	Root	Stem	Leaf	Seed	Leaf	Leaf
Atropine	0.0572	0.0768	0.0314	0.0876	0.074	0.0188	0.044	0.0904	0.1204
Scopolamine	0.00168	0.018	0.0236	0.00312	0.00276	0.00232	0.0252	0.0472	0.0139

Table 3. Linear regression equation and correlation coefficient for atropine and scopolamine.

R^2	Linear regression equation y = ax + b	Compound
0.9999	X + 38917 15262	Atropine
0.9996	1736.2 X + 9705.9	Scopolamine

scopolamine in leaves, seeds, and roots of *A. belladonna*. Likewise Harborne and Khan observed the same quantitative difference in these secondary products in *A. acuminata* (Simola et al., 1988).

We found that the maximum amount of atropine was present in the roots and stem (Table 2), which had a 4.6 and 2.4-fold higher quantity of this compound than recorded in leaves, for cultivated and native species, respectively.Scopolamine in the seeds was only 1.7-fold higher compared to leaves for cultivated species. The lowest level of this alkaloid occurred in roots and leaves for native and cultivated species tissue, respectively.

Linear regression equation and correlation coefficient for atropine and scopolamine was also determined (Table 3). Several reports in the literature indicate that the site of tropane alkaloid synthesis in a number of *Atropa* species lies in the roots (Hartmann et al., 1986).

Our data showed that *A. belladonna* and *A. acuminata* also constitutes the major storage site and had the highest amount of these alkaloids. These alkaloids may be transported to other plant organs providing potential deterrent and antimicrobial activities.

REFERENCES

Ballbach RL, Brown DJ, Walters SM (1977). Determination of diphenoxylate hydrochloride and atropine sulfate in solutions and tablets. J. Pharm. Sci., 66: 1553-1556.

British Pharmacopoeia (1993a). Vol. I, Her Majesty's Stationery Office, London.

British Pharmacopoeia (1993b). Vol. II, Her Majesty's Stationery Office, (1993), London. El-Masry S., Khalil S.A.H., (1973), J. Pharm. Sci., p. 62.

Evans WC (1979). Tropane Alkaloids of the Solanaceae, in Hawkes J. G., Lester R.N., Skelding A. D. (eds.), The Biology and Taxonomy of the Solanaceae. Linnean Society Symposium Series No. 7. Academic Press. London, pp. 241-254,

Gfeller JC, Frey G, Huen JM, Thevenin JP (1979). Post-column derivatization using the fluorometric ion-pair technique. J. High Resolut. Chromatogr., 1(4): 213-214.

Hassan SM, Davidson AG (1984). The assay of tropane derivatives in formulations by second derivative ultraviolet spectrophotometry. J. Pharm. Pharmacol., 36: 7-10.

Kamada H, Okamura N, Satake M, Harada H, Shimomura K (1986). Alkaloid production by hairy root cultures in Atropa belladonna. Plant Cell Rep., 5: 239-242.

Lund U, Hansen SH (1978). High-performance liquid chromatography on dynamically modified silica. J. Chromatogr., 161: 371.

Majlat P (1982). Gas chromatographic assay of atropine and phenobarbital in pharmaceutical preparations containing Valerian liquid extract. J. Chromatogr., 241: 399.

Pennington LJ, Schmidt WF (1982). Belladonna alkaloids and phenobarbital combination pharmaceuticals. J. Pharm. Sci., p. 71.

Reynolds JEF, Martindale (1993). The Extra Pharmacopoeia, The Pharmaceutical Press, London, 30th ed.

The United States Pharmacopeia (1995). United States Pharmacopeial Convention 23th revision.

Simola LK, Nieminen S, Huhtikangas A, Ylinene M, Naaranlahti T, Lounasmaa M (1988). Tropane alkaloids from Atropa belladonna, part II. Interaction of origin, age, and environment in alkaloid production of callus cultures. J. Nat. Prod., 51: 234-242.

Tyler VE, Brady LR, Robbers JE (1988). Pharmacognosy, 9th ed. Lea and Febiger , Philadelphia.

Waterman PG (1993). Alkaloids: general observations, pp. 1-16, *in* Dey P. M., Harborne J. B. (eds.), Methods in plant Biochemistry, Vol. 8, Alkaloids and Sulphur compounds. Academic Press, New York.

Willaman JJ, Li HL (1970). Alkaloid bearing plants and their contained alkaloids. J. Nat. Prod., 30: 1-4.