ISSN 0974 – 5211

Journal of Natural Products
(Only ON-LINE, ANNUAL, Science Journal published from INDIA)
Volume 9 (2016)
www.JournalofNaturalProducts.com

Research Paper

Quantitative identification of Atropine and Scopolamine in wild Datura (*Datura metel*) using HPLC

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(Received 29 Dec.. 2015; Revised 10 March-4 April 2016; Accepted 10 April 2016; On-lined 28 May 2016)

ABSTRACT

The purpose of this study was to determine the atropine and scopolamine content in different parts of Datura plant, (*Datura metel* L.), growing wild in Syria. These alkaloids were separated and quantified by high-performance liquid chromatography (HPLC) coupled with diode-array detector (DAD), and fluorescence detector (FLD), using a reversed phase Eclipse C_{18} column and employing a mixture of water with Acetic acid (2%): Methanol: Acetonitrile (10:80:10 v/v) as a mobile phase. Results showed that, the content of those two alkaloids in Datura varied according to season, location and plant tissue. The highest atropine concentration was found in the roots during autumn in plants collected from Damascus location then Lattakia and Banyas, (4900, 4700 and 4200mg/kg dry weight), respectively. The highest concentration of scopolamine was in plant flowers (4130, 3800 and 3430mg/kg dry weight for plants collected from Damascus, Banyas and Lattakia, respectively). While the lowest concentration of scopolamine was in plant seeds. The lowest atropine concentration was in the plant stem (1200-1400 mg/kg dry weight). In all samples the concentration of atropine was higher than scopolamine concentration except for flowers. Significant differences in atropine and scopolamine concentrations were found in plant parts on the level of per site as determined by LSD test at $P<0.05$.

Key words: *D. metel*; Atropine; Scopolamine; HPLC.

INTRODUCTION

Datura metel L., (*Solanaceae* family), is widely distributed plant in all the warmer parts of the world and is cultivated worldwide for its chemical and ornamental properties (Preissel et al., 2002) and it is also grown for its spiritual values (Jyothi and Taskeen, 2013). The various parts of the plant (leaves, seeds, roots and fruits) are used in medicine for different purposes. It is popular all over the world for its Insecticidal, Herbicidal, Anti-fungal, Antibacterial, Anti-cancer, Anaesthetic, Anti-asthmatic, Anti-spasmodic, Anti-tussive, Hallucinogenic, Hypnotic, Mydriatic, Anti-inflammatory and Anti-rheumatoid, activity. It was first described by Linnaeus in 1753 (Monira and Munan, 2012).

In Chinese medicine, it is used for the treatment of coughs, asthma, rheumatism, pain, and convulsions for centuries (Kuang et al., 2011; Pan et al., 2007). It also has an effect on the treatment of psoriasis for clinical use in China was used in a application at the First Affiliated Hospital of Heilongjiang University (Guarrera, 1999; Tang et al., 2006; Wang, 1985; Wang et al., 2008). It is used in Italy to remove lice from hen bundles (Maheshwari et al., 2013).

In chemical and pharmaceutical researches, *D. metel* is well known source for the production of tropane alkaloids such as hyoscyamine, scopolamine, anisodamine and anisodine (Zhong, 1986), flavonoids, phenols, tannins, saponins and sterols (Chopra et al., 1956; Oliver-Bever, 1986). Recently, withanolide compounds, the phytoconstituents of Datura, were analysed from various parts of the plant like the leaf (Anju and Ratan, 2011; Okwu and Igara, 2009; Kutama et al., 2010), root (Bhuktar et al., 2011), and shoot (Akharaiyi, 2011; Javaid et al., 2008).

Atropine and scopolamine are tropane alkaloids found in several members of the *Solanaceae* family, (Reynolds and Martindale, 1993). They also found in members of other plant families for example, *Erythroxylaceae, Convolvulaceae, Proteaceae, Orchidaceae, Euphorbiaceae, Cruciferae, Rhizophoraceae* (Evans, 1979) and in the fungus *Amanita muscaria* (Willaman and Li, 1970), and have anticholinergic properties and have legitimate medical applications in very low doses. Atropine, has a molecular mass of 289.37. Generally, atropine causes blurred vision, suppressed salivation, vasodilation increased heart rate, and delirium (Bruneton, 1999; Van wyk et al., 1997; Van wyk and Gericke, 2000). It also reduces rigidity in parkinsonism and is used as an antidote to poisoning with para sympathomimetic agents, e.g. nerve gases and organophosphorus insecticides (Dictionary of Natural Products, 2003). Scopolamine has a molecular mass of 303.36. Scopolamine is an antimuscarinic agent (used as an analgesic) and a smooth muscle relaxant. It is also an antispasmodic agent with antinauseant properties, and is extensively used in the treatment of motion sickness and in pre-operative medication (Van wyk et al., 1997; Van wyk et al., 2002).

Because there is no reports about atropine and scopolamine concentrations in *Datura metel* growing wild in Syria. This is the first study conducted in Syria to determine these compounds in Datura different plant parts, (leaves, flowers, stems, roots and seeds), using high performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Sampling: *Datura metel* L., samples were harvested in 2013 from three wild plant populations: The first sample was from sites located in Lattakia city (coastal region), the second were from sites located in Tartus city (Banyas-coastal region), and the third sample was from Yafoor site located in Damascus (semi-arid region). The plants were identified by Prof. M. Oudat (taxonomist, AECS). Voucher specimens have been deposited in the laboratory of the plant biotechnology department at the Atomic Energy Commission of Syria (AECS). From each collection site three separate individual plants were collected, from each individual plant leaves, stems, roots, flowers and fruits (seeds) were sampled. The raw materials were cleaned and oven dried at 40°C, till constant weight achieved.

Samples extraction: The dried samples extracted according to Agilent Technologies recommended method. Briefly, 1g of the dried and powdered plant was refluxed for 30min in 25ml acetic acid. After cooling, pH was adjusted to 9 and the solution was extracted three times with 50ml chloroform. After drying over sodium sulfate the

solvent was removed i. vac. and the residue dissolved in 2ml methanol. After filtration 20µl of the extract were applied to HPLC.

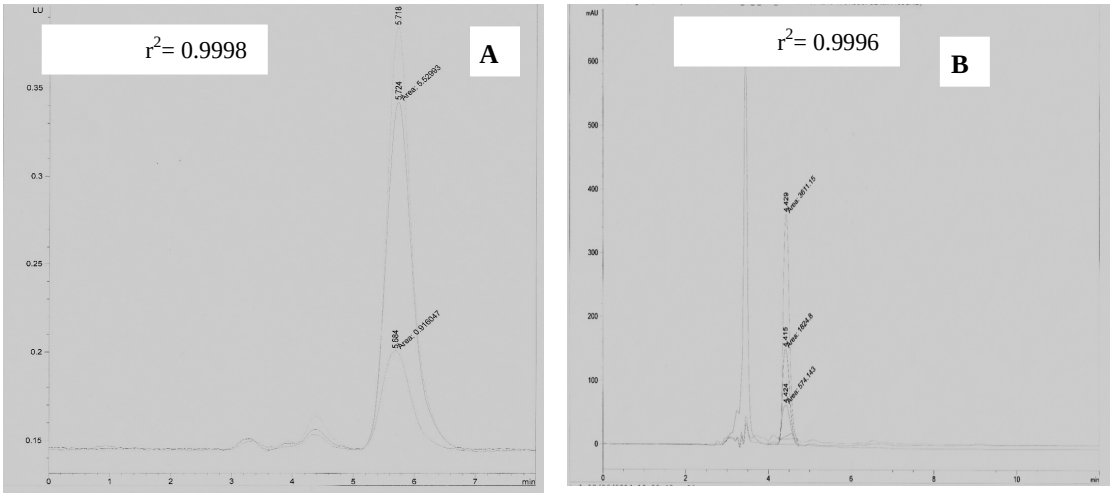
Chemicals, Standards solution: Atropine (99%) and scopolamine (98%) were purchased from Sigma-Aldrich. Other solvents and reagent was purchased from Merck. From methanolic stock solution of atropine and scopolamine (each 10mg/ml), standard solutions were prepared for the calibration. The standard working solutions used to build calibration curve were prepared by serial dilutions: 500, 800 and 1000ng/ml for Atropine and 1, 5 and 10µg/ml for scopolamine of stock solution with methanol. The solutions were stored at 4°C and were used for a week.

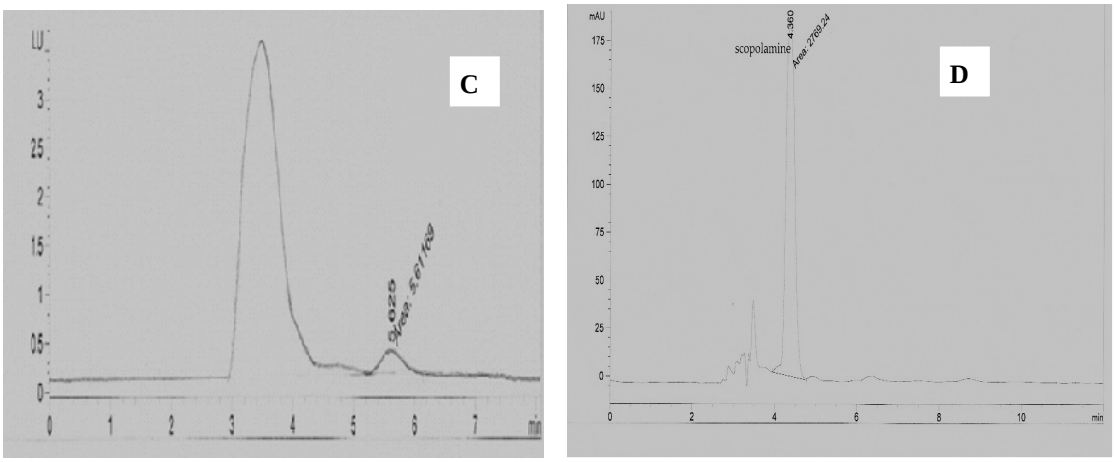
***High performance liquid chromatography (HPLC)*:** Chromatographic separation was achieved with LC system from Agilent (Infinity 1260) equipped with a diode array detector (DAD), and fluorescence detector (FLD), using a reversed phase Eclipse C_{18} column (150×4.6mm i.d.; 3.5µm) from Agilent Co. HPLC parameters for Atropine separation were as follows: Detector used was FLD with Excitation (EX) and Emission (Em) wavelengths at 255nm and 285nm respectively. The mobile phase was a mixture of Water with Acetic acid 2%: Methanol: Acetonitrile at volumetric ratio 10:80:10. Flow rate was 1.5ml/min. HPLC parameters for Scopolamine separation were as follows: Detector used was DAD at 210nm. The mobile phase was a mixture of Water with Acetic acid 2%: Methanol: Acetonitrile at volumetric ratio 10:80:10. Flow rate was 1.5ml/min. The calibration curves for atropine and scopolamine were constructed by plotting the peak area of alkaloids versus their concentrations.

***Statistical Analysis*:** Data were expressed as mean. One way analysis of variance (ANOVA) was used to asses the significance of differences among variables. SPSS softwear (version, 17) was used to perform multiple comparison tests by applying the least significant difference LSD at *P* values less than 0.05. Microsoft Excel program was also used to generate statistical histograms.

RESULTS AND DISCUSSION

Figure 1 shows typical HPLC chromatograms for standard materials of atropine and scopolamine. The retention times for Atropine (A) was 5.684–5.724min, and for scopolamine (B) was 4.415–4.429min. While the retention times for atropine and scopolamine (C, D) in analytical samples were 5.625, 3.60min, respectively. A good correlation of linearity has been achieved (n=3; $_R^2$ = 0.9998 and 0.9996) and, in the range of 500–1000ng/ml for atropine, and in the range of 1–10µg/ml for scopolamine. Limits of detection (LOD) and Limits of quantification (LOQ) values are summarized in table 1.





Retention time (min)

Figure-1: Typical HPLC chromatograms of chemical standards and samples.

- A: chemical standards for atropine ; B: chemical standards for scopolamine;
  C: Chromatogram of atropine in the leaves of *D. metel;*
  D: Chromatogram of scopolamine in the leaves of *D. metel* (t_R = 5.625 min; 4.360min).

Concentrations of atropine and scopolamine in leaves, roots, stems, flowers are given in table 1 and 2. The results of quantitative analysis of those main tropane alkaloids in *D. metel* plant tissues showed a significantly higher content of atropine than scopolamine in all samples studied except for flowers.

Table-1: LOD and LOQ results for atropine and scopolamine.

Sample	LOD pg/ml	LOQ (pg/ml)
Atropine	82.5	295
Scopolamine	180.5	600

Table-2: Amount of atropine determined in different tissues of *D. metel* (mg/kg) dry weight.

		Lattakia	Banyas	Damascus
Leaves	**Spring**	3200	2800	4100
	Summer	2910	2400	3700
	Autumn	2900	2300	3500
Stems	**Spring**	1300	1200	1400
	Summer	1300	1200	1400
	Autumn	1200	800	1200
Roots	**Spring**	4200	3900	4700
	Summer	4000	3700	4300
	Autumn	4700	4200	4900
Seeds	**Autumn**	1900	1700	2200
Flowers	**Summer**	2700	2430	3200

- Atropine concentration expressed as mg/g derived from the average of three independently extraction replicates.

The results showed that, atropine and scopolamine concentration in Datura varies according to the organ studied, and geographical location. We found that the maximum amounts of atropine were in roots and leaves (**Table-2**). Atropine concentrations ranged from 3700–4900 mg/g DW in roots and 2400–4100 mg/g DW in leaves. Lesser concentrations of atropine were measured in flowers, seeds and stems. The maximum amounts of scopolamine were in flowers, than in roots and

leaves. The lowest concentration of this alkaloid was in seeds and stems (**Table-3**). Scopolamine concentration in flowers was 2.5-3.2 times higher than that in leaves, and 5.2-7.6 times higher than that measured in stem samples. This concentration was 1.1-1.4 times higher than that measured in roots during the flowering season (in summer).

Table-3: Amount of scopolamine determined in different tissues of *D. metel* (mg/kg) dry weight.

		Lattakia	Banyas	Damascus
Leaves	**Spring**	**1500**	**1400**	**1900**
	Summer	1400	1200	1700
	Autumn	1400	1200	1600
Stems	**Spring**	500	600	800
	Summer	500	500	800
	Autumn	300	500	600
Roots	**Spring**	3100	2700	3400
	Summer	3100	2700	3400
	Autumn	3600	2900	3800
Seeds	**Autumn**	400	300	500
Flowers	**Summer**	3430	3800	4130

- Scopolamine concentration expressed as mg/g derived from the average of three independently extraction replicates.

Atropine and scopolamine concentrations in plant samples from different locations ranged from 2300 to 4100mg/g DW for atropine and from 1200 to 1900 mg/g DW for scopolamine in leaves. In roots those concentrations ranged from 3700 to 4900 mg/kg DW for atropine and from 2.7 to 3.8mg/g DW for scopolamine. While in flowers they ranged from 2430 to 3200mg/kg DW for atropine and 3.43 to 4.13mg/g DW for scopolamine. In stems and seeds, the concentrations of those alkaloids were generally low compared with other plant tissues. The concentration of atropine and scopolamine measured in samples from Damascus location was higher than those determined in samples from Banyas and Lattakia locations (**Table-2, 3**).

Analysis of variance using LSD test at $P<0.05$ showed statistically significant differences in atropine and scopolamine concentration between all plants parts in the same location, and significant differences in atropine concentration in leaves sampled during spring, and flowers sampled during summer. A significant differences were also found for scopolamine concentrations in roots sampled during summer and autumn between the various sampling locations ($P<0.05$). However, stems and seeds samples showed no significant differences in atropine and scopolamine concentrations between the various sampling locations ($P>0.05$).

By comparing concentrations of atropine and scopolamine measured in the *Datura metel* from Syria with those reported for the same species by others, we find that those concentrations were relatively similar to those recorded for plants from India, Japan, and Poland. Atropine and scopolamine concentrations in leaves of *D. metel* growing wild in India ranged between 2200mg/kg DW for atropine and 1810mg/kg DW for scopolamine. Those concentrations were 3200 mg/kg DW for atropine and 1250mg/kg DW for scopolamine in seeds, a 3200mg/kg DW for atropine, and 5510mg/kg DW for scopolamine in flowers (Shah and Khanna, 1963). A concentration of 1910mg/kg DW for atropine and 1250mg/kg DW for scopolamine were also reported in seeds for the same species by Shah and Khanna (1964). In the leaves of *D. metel* var. fastusa, atropine and scopolamine concentrations were 3300mg/kg DW and 2610mg/kg DW, respectively. In the flowers of this var. those concentrations were 3210 and 4770mg/kg DW respectively (Shah and Khanna, 1963). Hiraoka et al., (1996) found that scopolamine concentration in leaves of *D. metel* var. muricata, growing wild in Japan, ranged between 810-820mg/kg DW. This

concentration ranged between 2280-2530mg/kg DW in the leaves of *D. metel* var. metel. Mroczek et al., (2006), found that scopolamine content in the seeds of *D. metel* var. fastuosa growing wild in Poland ranged between 756-773mg/kg DW.

Comparing the concentrations of atropine and scopolamine we found in *Datura metel* with those measured in on other species of Datura we notice a convergence in plants content of those alkaloids. However this convergence varys depending on the plant part studied, for example: The aerial parts of *D. stramonium* (leaves) contain mainly the atropine (Miraldi et al., 2001). The main alkaloid in seeds is also the atropine (Dugan et al., 1989; Klein-Schwartz and Oderda, 1984; List et al., 1979; Miraldi et al., 2001), although scopolamine is present in significant amounts (Miraldi et al., 2001). On the other hand, the flowers are characterized by the dominance of scopolamine 2700-2990mg/kg DW over atropine 660-1060mg/kg DW (Miraldi et al., 2001). In *D. innoxia* the leaves and stems contains mainly the atropine (340-1350mg/kg DW), and a lesser amount of scopolamine (110-369mg/kg DW). However; the seeds contained 26mg/kg DW of atropine and 29mg/kg DW of scopolamine (Galey et al., 1996).

Through the observation of the studies carried out on Datura which gives importance to the study of the extent of these compounds in spite of the different content depending on plant part studied and place of growing. It is likely that environmental factors such as rainfall, the altitude above sea level, soil composition and soil salinity in addition to phenological stage plays an important role in the differences seen in plant contents of those alkaloids. These prospects confirmed by other researchers (Afsharypuor et al., 1995; Berkov and Zayed, 2004; Miraldi et al., 2001; Shonle and Bergelson, 2000).

CONCLUSION

The alkaloid content of *D. metel* varies to a great extent depending on the plant part concerned. The root was the organ that accumulates the higher amounts of atropine, and the flowers the organ which accumulates the higher amounts of scopolamine. Atropine concentrations in *D. metel* parts were in root> leaf> flower >stem>seeds and for scopolamine the concentration was in flower> root> leaf> stem>seeds. The roots and aerial parts of Datura samples from Damascus location contained the highest concentrations of those two alkaloids, although they were found in significant amounts in plants collected from Lattakia and Banyes. Economically *D. metel* roots, flowers and leaves might be considered a cheap and wealthy source for atropine and scopolamine.

Acknowledgements: We express our thanks and gratitude to Prof. Ibrahim Othman, Director General of Atomic Energy Commission of Syria and Prof. Nizar Mir Ali for support and encouragement.

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