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COMPARATIVE SCREENING FOR DETERMINATION OF ATROPINE AND SCOPOLAMINE IN DIFFERENT SPECIES OF DATURA IN IRAQ AND IRAN

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ABSTRACT

This study aimed to inspect the presence of atropine and scopolamine alkaloids in two species of *Datura* (*D. stramonium* & *D. innoxia*) from different places in Iraq and Iran. The whole plant were collected, dried, mashed, and then extracted by using solvent system 15:15:1 (Chloroform/Methanol/Ammonia 25%) Determination of alkaloids was performed by high-performance liquid chromatography (HPLC) method using C18 column and isocratic mobile phase of acetonitrile-50 mM in phosphate buffer (pH 2.95). A comparative HPLC investigation of the alkaloid showed that there are significant differences in the quantities of the alkaloids in the plants of different origin, indicating that the influence may be attributed to the environmental factors.

Keywords: Species of *Datura*; atropine and scopolamine alkaloids; plants

Introduction

Natural products had played a great role in the growth of organic and medicinal chemistry, including fundamental aspects of stereochemistry, mechanistic chemistry, biosynthesis and mechanism of biological action, as well as providing medically beneficial compounds (Babiker *et al.*, 2017). Among natural products, the secondary metabolites, which give the species its characteristic features. Unlike primary metabolites, these compounds are unique in the living organisms that neither produce them nor are they expressed continuously. So, plants are the best-known sources (Jamal, 2016).

At the last times which is not's far away, the researchers concentrated on the investigation and identification of plant phytochemicals in order to use it as a potential source for therapeutic purposes. Plants are rich in different and high levels of secondary metabolites such as tannins, flavonoids, phenols, steroids, alkaloids, glycosides and volatile oils (Saxena *et al.*, 2013). Tropane alkaloids are bicyclic compounds found in different genera in the Solanaceae and Erythroxylaceae. These compounds work as anticholinergic agent's affecting the central and peripheral nervous system a competitive, non-selective muscarinic acetylcholine receptor antagonists that prevent the binding of the physiological neurotransmitter acetylcholine. The alkaloids atropine, scopolamine, and their derivatives are the active ingredients in numerous pharmaceuticals ranging from antidotes for poisoning by organophosphorous compounds,

antispasmodics, anti-motion sickness agents, and anti-emetics (Moreno-Pedraza *et al.*, 2019).

The genus *Datura* is a member of the Solanaceae family and it's included about 20 species which grow worldwide (Evans *et al.*, 1972). *Datura innoxia* Mill, Is one of them, its native in America, and now distributed widely in the warm regions of the world and its poisonous nature and noxious smell, maybe to defenses against herbivores (ElBazaouia *et al.*, 2012). Three species found in Iraq, *D. innoxia* Mill, *D. metel* L. and *D. stramonium* L. which are cultivated or may be found as escaper of cultivation here and there (Chakravarty, 1976). All parts of *Datura* plants are toxic; containing dangerous levels of tropane alkaloids and can cause hallucinations and may be fatal if ingested by humans and other animals (Barguil *et al.*, 2006).

This study was carried out for the determination and quantification of alkaloids compounds of *Datura* species growing in north of Iraq and compare it with Iranian species by using HPLC analytical methods.

Material and Methods

Samples collection

An intensive survey was carried out during July 2019 in the north of Iraq for the collection and identification of plants. The plant, *D. stramonium* were collected from Penjwin area and *D. innoxia* from two different places, Sulaimaniya and Balad in Iraq. Iranian samples were

collected during September 2019 from Tehran by corporation with Tehran University. The plant was washed to get rid of contaminants, and then the plant was air-dried in the shade for several days at room temperature and ground into a fine powder.

Chemical study

The Chemical study was carried out in the department of pharmacognosy and medicinal plants in college of pharmacy, University of Basra, Basra, Iraq.

Preparing materials

After plant collection, the plants are cleaned and dried under natural open air, then grinded and placed in plastic bags and labeled.

Alkaloids extraction

Alkaloids were extracted according to (Harbone, 1984). 20 gm of a plant powder were taken and extract with the solvent system (chloroform: Methanol: Ammonia 25%) (15:15:1) with stirring for about 1h at room temperature. The extracts then filtered and evaporated to dryness. The dry residue was dissolved in chloroform and acidified with sulfuric acid until the pH becomes around 3-4. Organic phase was discarded, and the aqueous one was alkalized by adding 25% NH₄OH on ice to make the pH around 10-12. The alkaloid were extracted twice with chloroform. After that addition of anhydrous Na₂SO₄, filtrated and the residue washed with chloroform then the solvent was evaporated to dryness under vacuum at 40°C

Tests of Alkaloids :

All tests was done according to description of (Harborne, 1984).

Wagner's test

1 ml of Wagner's reagent was added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids.

Dragendoff's test

1 ml of Dragendoff's reagent was added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids.

Mayer's test

1 ml of Mayer's reagent was added drop by drop. Formation of greenish color or cream precipitate indicates the presence of alkaloids.

(HPLC) analysis

Alkaloid extraction was performed essentially as described by (Ashtiani & Sefidkon, 2011). 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 Imax and the column used was packed with 25 x 0.46 cm Eurospher- 100 C8 (Knauer, Germany, A) and Lichrospher 100 RP8 (Teknokroma, Spain, B), packed 5 µm particles.

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.

Results and Discussion

After the successful extraction of the whole part of the plant in the investigation, the preliminary phytochemical study revealed that the methanolic extract of *D. spp.* contains alkaloids and gave positive results for all tests as shown in table 1.

Table 1: Phytochemical Screening of *Datura* spp.

Test	Iraqi <i>D. stramonium</i>	Iraqi <i>D. innoxia</i>		Iranian <i>D. stramonium</i>	Iranian <i>D. innoxia</i>
		Sulaimaniya	Balad		
Wagner	+	+	+	+	+
Dragendoff	+	+	+	+	+
Mayer	+	+	+	+	+

The results of the HPLC analysis of the *Datura* species from Iraq and Iran lead to the identification of a number of tropane and scopolamine compounds in different concentration as bellow:

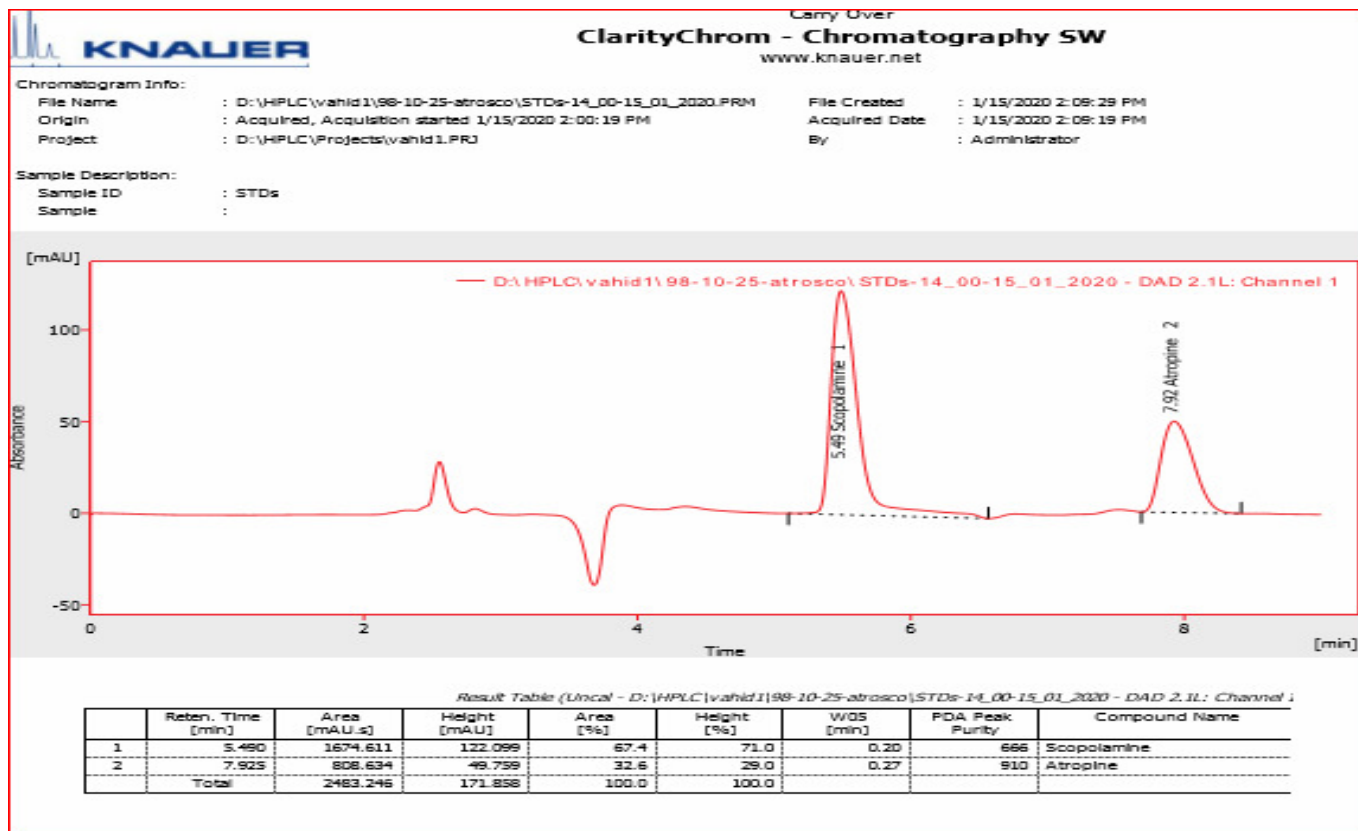


Fig. 1 : HPLC chromatogram for standards

In Scopolamine standard chromatogram, the sample observed around the minute 5.490, while atropine observed in minute 7.925 as shown in fig 1 and table 2. In methanolic extract of *Datura* spp. we observed an absorption peaks for scopolamine ranging between minute 5.406 to 5.790, while atropine ranging between minute 7.660 to 7.832 as shown in figures 2,3,4 and table 3.

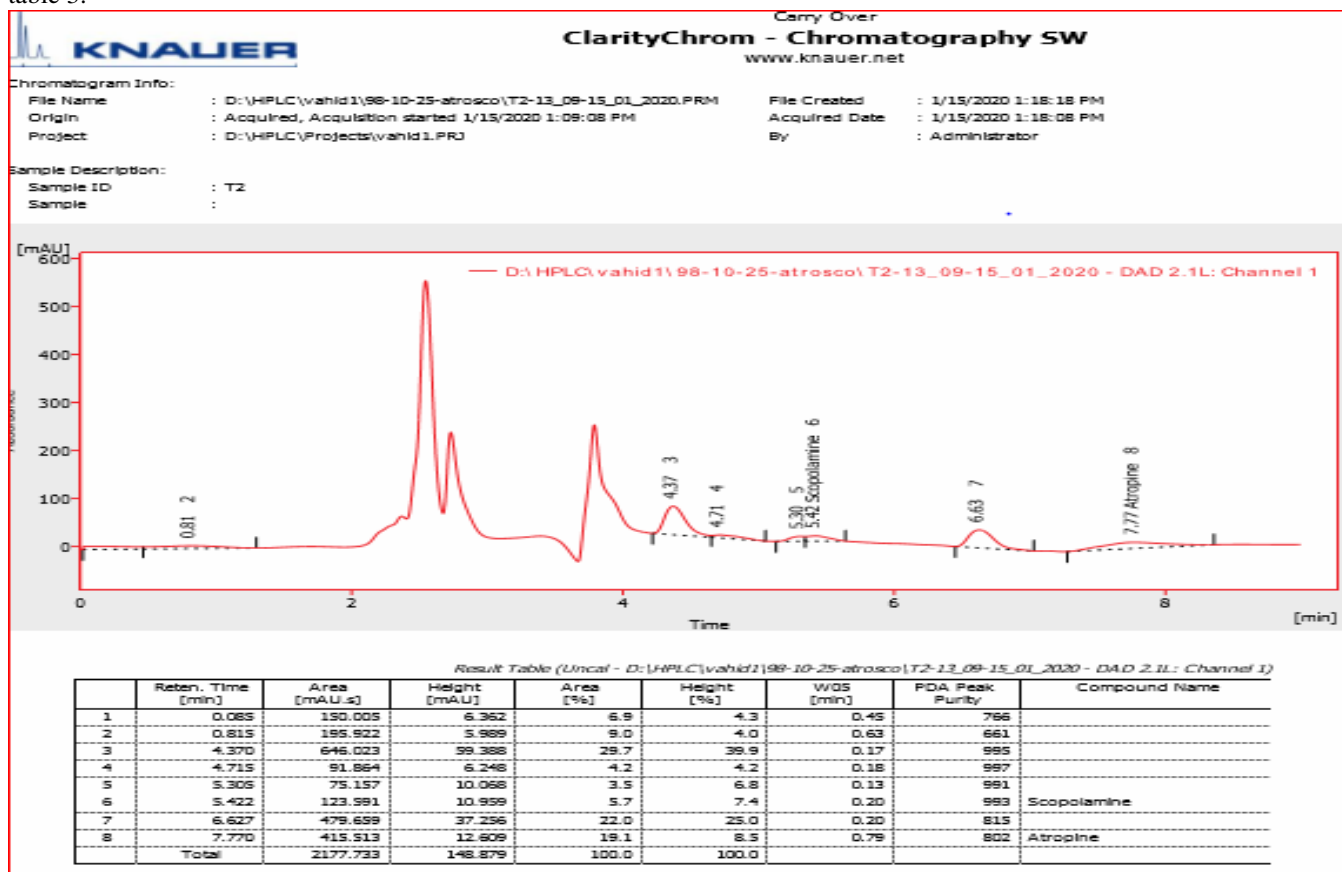


Fig. 2 : HPLC chromatogram for *D. stramonium* from A (Penjwin-Iraq) and B (Iran).

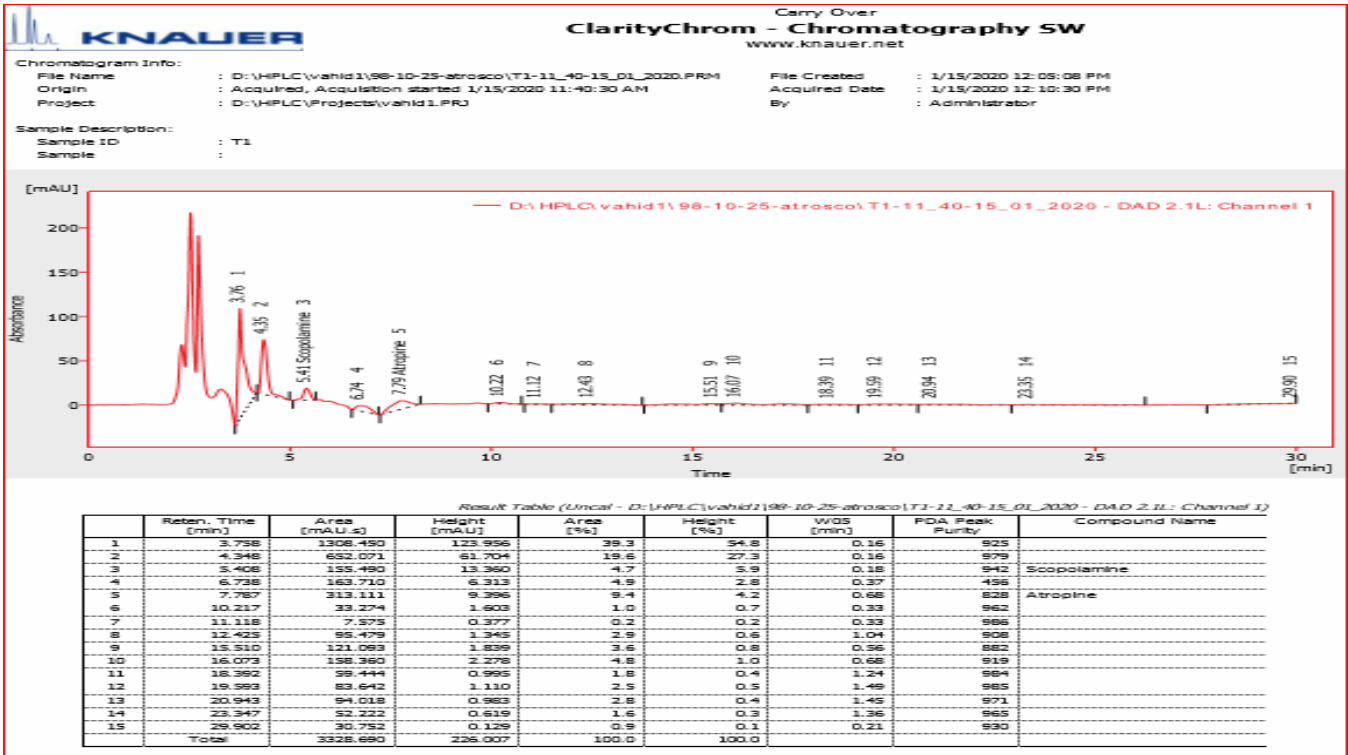


Fig. 3 : HPLC chromatogram for *innoxia D.* from Sulaimaniya (C) and Balad (D)

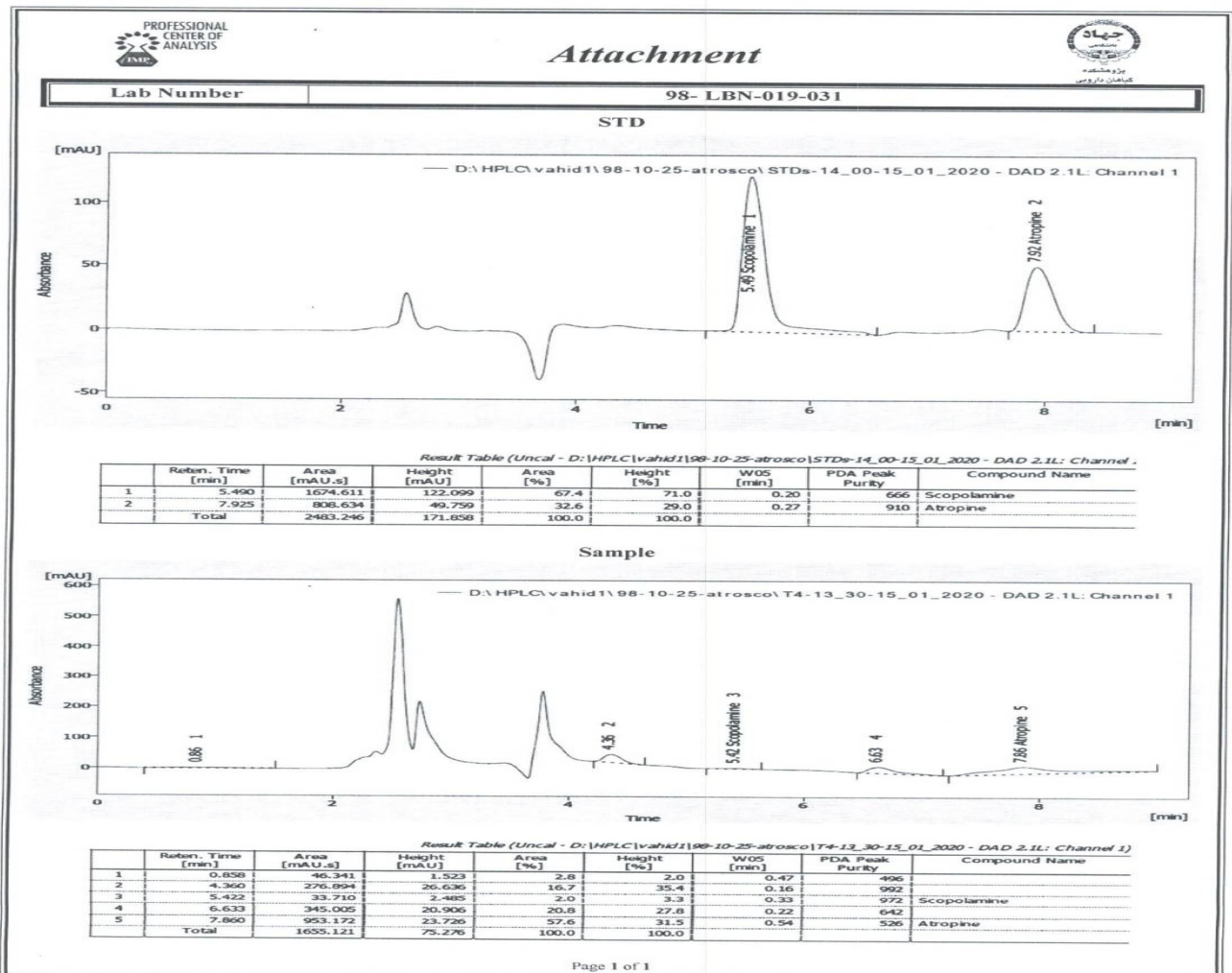


Fig. 4 : HPLC chromatogram for *D. innoxia* from Iran.

Table 2 : The scopolamine & atropine quantity in the *Datura* spp. samples

<i>Datura</i> spp.	Reten. time (min) Scopolamine	Reten. time (min) Atropine	Percentage For Scopolamine	Percentage For Atropine
<i>D. innoxia/ Sulaimaniya</i>	5.472	7.770	0.369	1.285
<i>D. innoxia/ Balad</i>	5.406	7.707	0.489	0.968
<i>D. innoxia / Iran</i>	5.790	7.832	0.214	0.902
<i>D. stramonium/Penjwin</i>	5.415	7.822	0.081	1.144
<i>D. stramonium / Iran.</i>	5.422	7.660	0.101	2.947

Quantitative analysis of atropine and scopolamine were determined on various species of *D. innoxia* and *D. stramonium* from different places in Iraq and compared it with Iranian species. The results have been summarized in table 3 and figure 4, where the amount of atropine alkaloids appears more than the scopolamine in all species and places. While the percentage of scopolamine in Iraqi species is more than in Iranian species. Our findings indicated that the distribution of atropine alkaloids and scopolamine was different from place to place and this because of the variation in the environment.

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