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Identification and Determination of Concentrations of some Toxic Alkaloids in Jimsonweed (Datura stramonium L.)

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ABSTRACT
Alkaloids are regarded as complex naturally occurring products of plants and fungi. They possess nitrogen-containing heterocyclic rings, basic and derived from amino acids. High physiological activity is also a characteristic of alkaloids. This study was aimed to determine the existence and concentrations of some toxic alkaloids in different parts of jimsonweed (*Datura stramonium* L.) of the family Solanaceae. Stems, leaves and fruits were dried, crushed and then underwent extraction. Solvents used in the extraction were chloroform and acetic acid. Thin layer chromatography (TLC) and GC-MS techniques were also adopted in analyzing the alkaloids. The results revealed the presence of atropine in stems, leaves and fruits of *D. stramonium* with concentrations of 0.03, 0.4 and 0.08 µg/g, respectively. Hyoscine was also found in stems, leaves and fruits with concentrations of 0.01, 0.02 and 0.2 µg/g, respectively. Nicotine was found in stems and fruits with concentrations of 0.001 and 0.02 µg/g, respectively. This study showed and provided the possibility of exploring new natural potential pesticides from Sudanese indigenous plants and provide important information to avoid toxicity, harmful and adverse effects of these plants to both humans and animals.

KEYWORDS Alkaloids, Solanaceae, jimsonweed, GC-MS techniques, Nicotine, atropine.

1. INTRODUCTION

Solanaceae is one of the largest families which consists of about 98 genera and 2700 species (Olmstead and Bohs, 2007). Most of the plants in this family contain a large number of alkaloids, which are highly toxic chemicals and many have serious effects on humans and animals (Olmstead *et al.*, 1999). Although alkaloids have high toxicity, but many alkaloids have medical and industrial uses such as atropine, hyoscine, nicotine, solanine and others (Szymon *et al.*, 2016). Many alkaloids can be purified from crude extracts by acid-base extraction and are very toxic to other organisms. They often have pharmacological effects and are used as medications as recreational drug, or in entheogenic rituals (Tarek, 1997).

Atropine and hyoscine are considered the most common alkaloids due to their high toxicity and their effect on the nervous system. In addition to their various medical uses, the main source of these two types of alkaloids are *Datura* spp. The main source of nicotine are *Nicotiana* spp. Solanine, which is found in potato and eggplant, is highly toxic and has a number of medical and industrial uses. The first complete synthesis of alkaloids was achieved in 1886 by the German chemist Albert Ladenburg who produced coniine. The development of the chemistry of alkaloids was accelerated by the emergence of spectroscopical and chromatographic methods in the 20th century and by 2008 more than 12000 alkaloids were identified (Sazima*et al.,* 2003).

Objective of the study

The objective of this study was to determine the existence and concentrations of some alkaloids in jimsonweed (*Datura stramonium* L.).

2. MATERIALS AND METHODS

This study was conducted in laboratories of General Administration of Forensic Evidences (GAFE) Khartoum,Sudan and the Department of Pesticides and Toxicology, Faculty of Agricultural Sciences, University of Gezira, Wad Medani, Sudan from January 2018 to January 2019.

Samples preparation

Stems leaves andfruits of Datura were collected from the Eastern area of Wadmedani town and then dried in an oven at 80 C^0 for 48 hours

Preparation of standard samples

Standard samples of atropine, hyosine, nicotine and solanine were purchased from Sigma Aldrich Company, USA. Atropine standard was prepared by adding 1 ml of chloroform to 1 g of atropine. Hyoscine and nicotine standards were prepared by adding 1 ml of chloroform to 1 ml of hyoscine and 1 ml of nicotine, while solanine standard was prepared by adding 1 ml of acetic acid to 1 g of solanine.

Extraction

Five ml of chloroform was added to 1.0 g of herbal powder of each plant part and left for 24 hours at room temperature, and then the suspension was filtered using filter paper in the second day (Moffat *et al.*, 2011). Regarding solanine, 5 ml of diluted acetic acid was added to 1.0 g of each plant part and kept for 24 hours at room temperature. The obtained suspension was filtered by using filter paper in the second day (Moffat *et a*l., 2011).

Thin layer chromatography (TLC)

Samples of each plant part were loaded in TLC plate and a control sample of atropine, hyoscine, nicotine and solanine were loaded in the plate beside the plant samples, and each plate was used for the detection of one type of targeted alkaloids. The Retention Factor (RF) was calculated according to the following equation:

$$R\,f \text{ value} = \frac{\text{Distance from baseline travelled by solute}}{\text{Distance from baseline travelled by solvent (Solvent front)}}$$

The RF was observed under ultraviolet radiation at a wavelength of 254 nm (Moffat *et al.,* 2011).

Gas chromatography analysis with mass spectrometer (GC- MS)

Samples with positive results were injected in GS-MS. The injection volume was 1 µl and the start temperature of the column was 80 ^{O}C held for 1 minute, then increased to 200 ^{O}C, 260 ^{O}C at the rate of 15 degree and 10 degree per minute, respectively. The last increase was to 290 ^{O}C at the rate of 10 degrees but for 2 minutes. The results were shown in chromatograms and block spectra and were compared to NIST (National Institute of Standards and Technology) library which was attached to the device.

3. RESULTS AND DISCUSSION

Result of TLC analysis

At 10 cm run, the mobile flow rates (RF) for the standard samples of atropine, hyoscine, nicotine and solanine were 0.2, 0.53, 0.36 and 0.23, respectively. The samples of the plant parts did not show positive results for solanine. This result is in agreement with that reported by Babiker *et al.* (2017). All plant parts except the root showed a positive result for atropine, hyoscine and nicotine.

Results of GC- MS analysis

Result of the standard samples

The chromatogram of atropine standard showed a sharp peak at the retention time (Rt) of 14.3 minutes as shown in Figure (1).

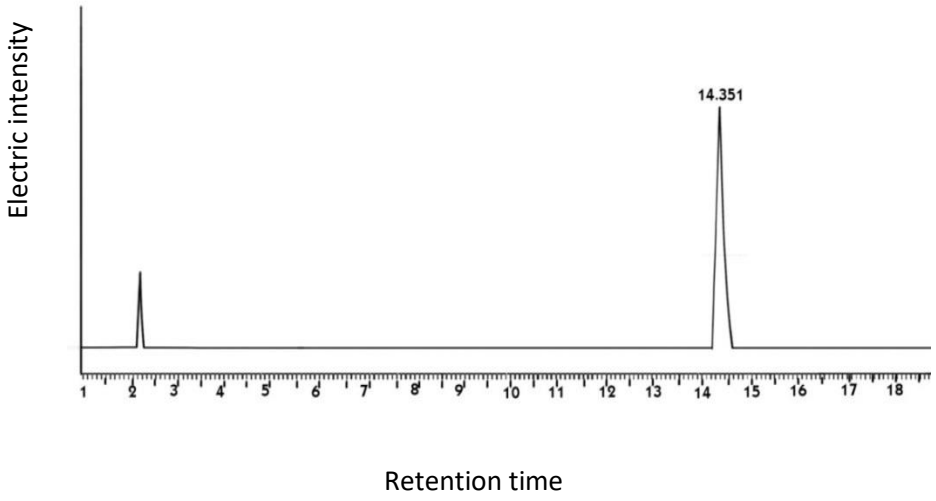

Figure 1. Standard atropine chromatogram

The main fragment peak mass value was 124, while the highest fragment mass was 289 (Figure 2).

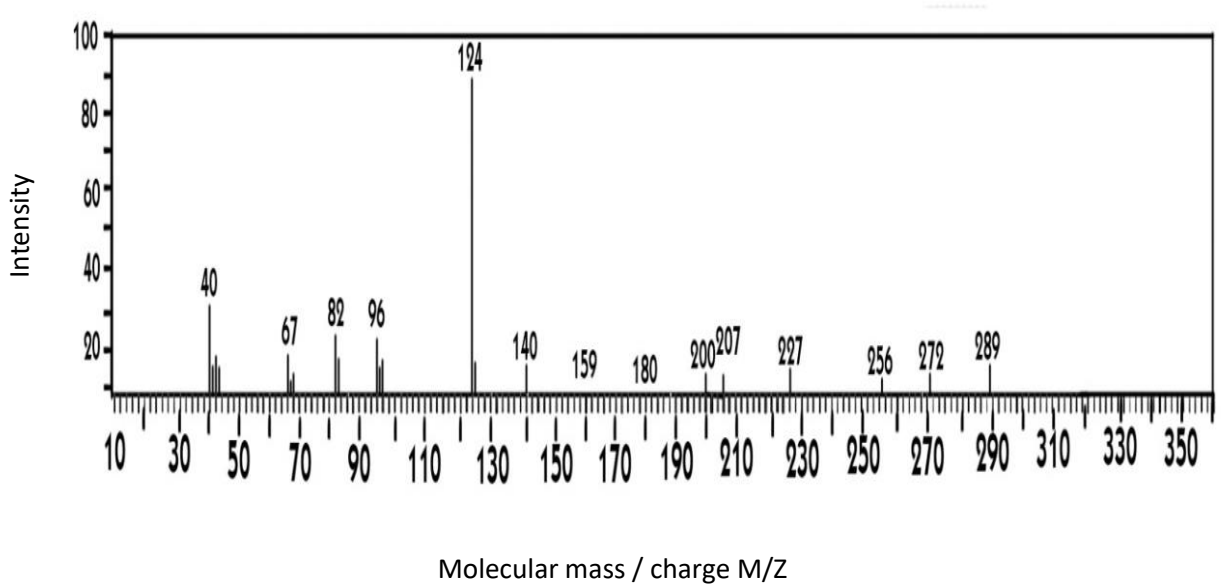

Figure 2. Mass spectrometer of the standard atropine

Solanine standard chromatogram showed a sharp peak at Rt of 7.6 minutes ,(Figure 3).

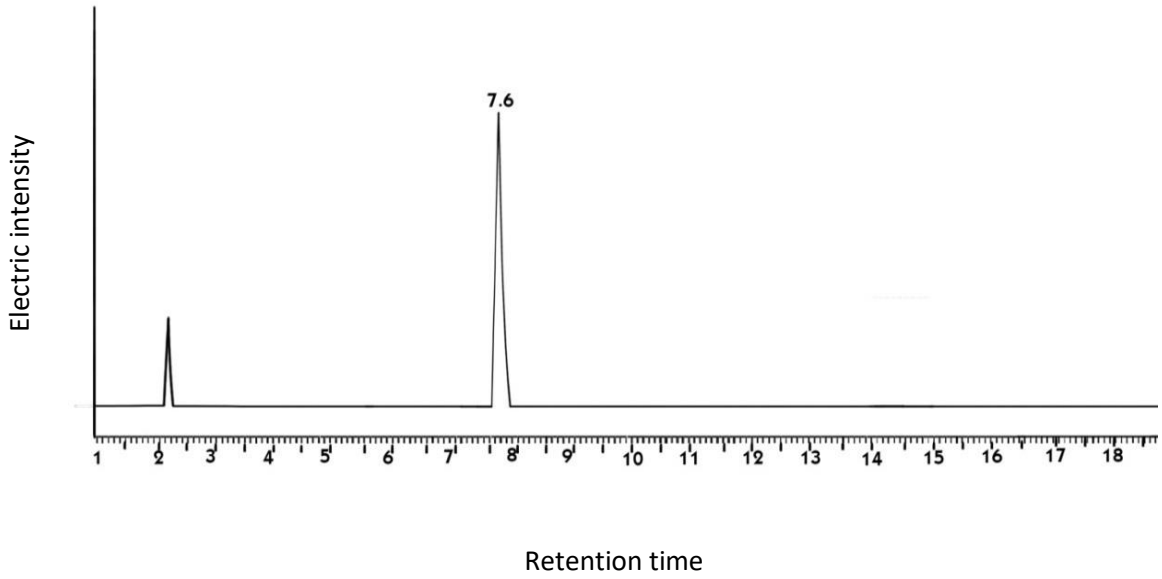

Figure 3. Standard solanine chromatogram

The highest mass value of the mass spectra of these peaks was 868 as well as it is the main fragment peak as shown in Figure (4).

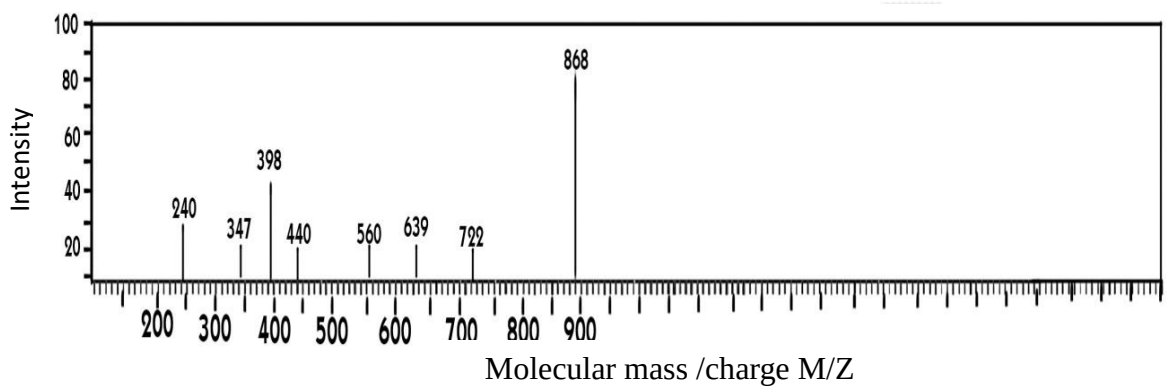

Figure 4. Mass spectrometer of solanine standard

For nicotine, the Rt of its peak was 6.3 minutes as shown in Figure (5).

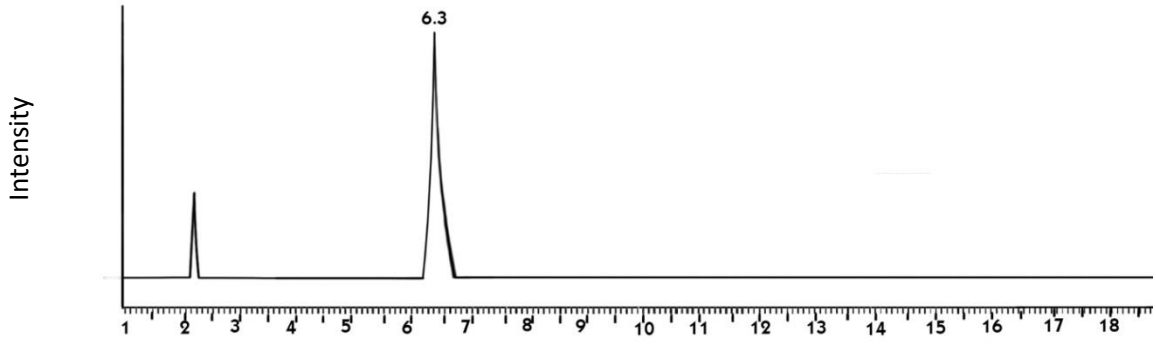

Figure 5. Standard nicotine chromatogram

The highest mass fragment value of the mass spectra was 163 and the main fragment mass was 84 (Figure 6).

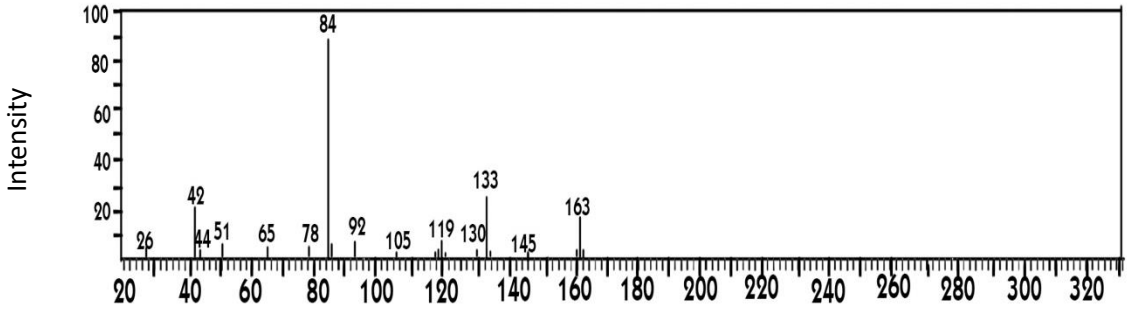

Figure 6. Mass spectrometer of standard nicotine

Hyoscine standard, peak appeared in the chromatogram at Rt of 14.5 minutes (Figure 7).

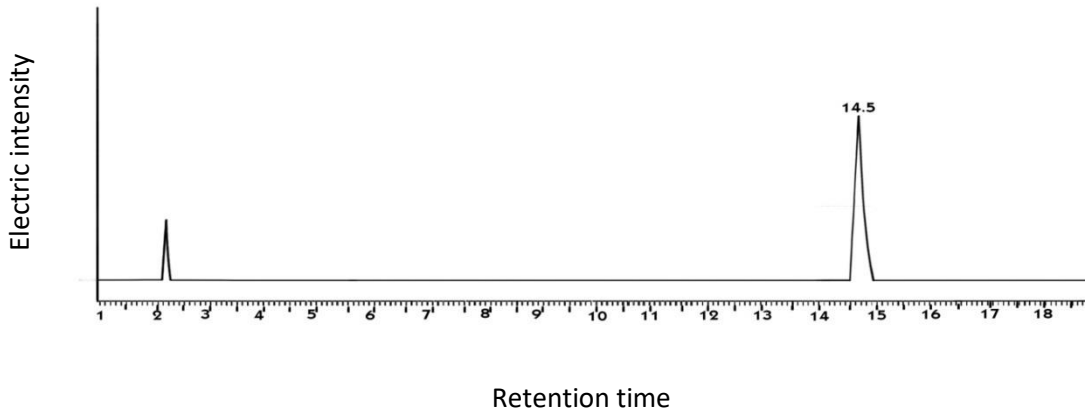

Figure 7. Standard hyoscine chromatogram

For hyoscine standard, the main fragment mass value was 124 while the highest mass fragment value was 303 (Figure 8).

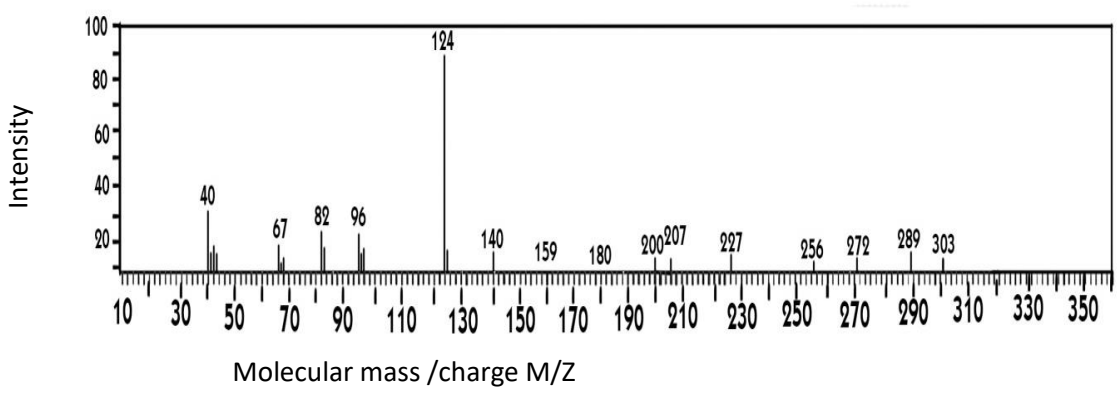

Figure 8. Mass spectrometer of the standard hyoscine

Results of GC -MS analysis for *D. stramonium*

The chromatogram of stem sample of *D. stramonium*showed a peak at 14.3 minutes. The mass spectra of this peak showed the molecular peak value of 289 and base peak value124 (Table 1). The other fragment in the mass spectra was consistent with what was obtained from the mass spectra of the standard sample of atropine which is confirming the presence of atropine in the sample. This mass spectrum was compared to internal library and the similarity was 87%. This result confirmed the existence of atropine in the sample. The same sample chromatogram showed a peak at 14.5 minutes, the mass spectra of molecular peak was 303 and base peak value was 124. The other fragment of this mass spectra were consistent with the mass spectra of hyoscine standard sample and the similarity in the internal library was 88%, which showed the

existence of hyoscine in the sample. In the same chromatogram, at 6.3 minutes a peak was observed and the mass spectra of this peak showed a molecular peak value of 163 and a base peak of 84 which is similar to the fragment of the mass spectra of the nicotine standard. The internal library showed a similarity of 77% which confirmed the existence of nicotine in the sample.

Table 1. GC-MS analysis results of the stem of *Daturastramonium*L.

Alkaloids	Retention time (Rt)	Molecular peak	Base peak	Similarity (Internal library)	Result
Atropine	14.3	289	124	87%	Confirming existence of atropine
Hyoscine	14.5	303	124	88%	Confirming existence of hyoscine
Nicotine	6.3	163	84	77%	Confirming existence of nicotine

The leaf sample of *D. stramonium* chromatogram showed a peak at 14.3 minute the mass spectra of this peak revealed the molecular peak value of 289 and base peak value 124 (Table 2). The other fragment in the mass spectra was consistent with what was obtained from the mass spectra of the standard sample of atropine. The similarity of the internal library was 79%. This result is confirming the existence of atropine in the sample. The same sample chromatogram showed a peak at 14.5 minutes, the mass spectra of this peak gave a molecular peak value equal to 303, and a base peak value was 124. The other fragment of this mass spectra were consistent with the mass spectra of the hyoscine standard sample.

Table 2. GC-MS analysis results of leaves of *Datura stramonium*L

Alkaloids	Retention time (Rt)	Molecular peak	Base peak	Similarity (Internal library)	Result
Atropine	14.3	289	124	79%	Confirming existence of atropine
Hyoscine	14.5	303	124	85%	Confirming existence of hyoscine
Nicotine	6.3	163	84	79%	Confirming existence of nicotine

Moreover, the similarity in the internal library was 85%, which confirmed the existence of hyoscine. In the same chromatogram, at 6.3 minutes a peak was observed. The mass spectra of this peak showed a molecular peak value equal to 163 and a base peak value of 84 which is similar to nicotine standard sample and the similarity in the internal library was 79%, which indicated the existence of nicotine in the sample.

The fruit sample of *D. stramonium* chromatogram showed a peak at 14.3 minute, (Table 3). The mass spectra of this peak reflected molecular peak value equal to 289 and a base value of 124. The other fragment in the mass spectra was consistent with what was obtained from the mass spectra of the standard sample of atropine. This mass spectrum was compared to the internal library and the similarity was 89%. This result confirmed the existence of atropine in the sample. The same chromatogram sample showed a peak at 14.5 minutes and the mass spectra of this peak gave a molecular peak value equal to 303, and the base peak value was 124. The other fragment of this mass spectra were consistent with the mass spectra of hyoscine in the standard sample. The similarity in the internal library was 83% which proved the existence of hyoscine in the sample. The result for nicotine was negative.

Table 3. GC-MS analysis results of fruits of *Daturastramonium*L.

Alkaloids	Retention time (Rt)	Molecular peak	Base peak	nilarity (Internal library)	Results
ropine	.3	9	4	%	Confirming existence of atropine
osine	.5	3	4	%	Confirming existence of hyoscine
cotine					No positive result for nicotine

The concentrations of atropine and hyocine in *D. stramonium* (Table 4), are consistent with the findings of Babiker*et al.* (2017). Nicotine was found only in the stem and leaf of Datura, but was not found in the fruit.

Table 4. Concentrations of alkaloids in*Daturastramonium*.

Plant part	Concentration (µg/g)		
	Atropine	Hyoscine	Nicotine
Stem	0.03	0.01	0.001
Leave	0.04	0.02	0.02
Fruit	0.08	0.2	-

DECLARATION

I declare that there is not any kind of conflict of interest among the authors of this paper.

CONCLUSION

The current study revealed that *D. stramomium* is a promising natural product to be used as a botanical pesticide and further studies are needed using various bioassay techniques to prove that.

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