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Artículos originales

Determination by High performance liquid chromatography and colorimetric of the alkaloids of *Hyoscyamus muticus* L. subsp *falezlez* (Coss.) Maire in three harvesting areas of the Algerian Sahara

Determinación mediante cromatografía líquida de alto rendimiento y colorimetría de los alcaloides de *Hyoscyamus muticus* L. subsp *falezlez* (Coss.) Maire en tres zonas de cosecha del Sahara argelino

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Conflict of interest

We declare that there are no conflicts of interest in relation to this document

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Resumen

Introducción: *Hyoscyamus muticus* L. subsp *falezlez* (Coss.) Maire es una especie sahariana rica en alcaloides tropánicos (especialmente hyoscyamine). La hiosciamina se convierte en atropina, cuyo interés en la farmacia es considerable.

El objetivo es dosificar alcaloides de *Hyoscyamus muticus* L. subsp *falezlez* (Coss.) Maire de las estaciones del Sahara argelino (Abadla, Adrar y Tamanrasset), para explotar los datos en la valorización de esta especie como fuente potencial de producción industrial de atropina.

Método: La determinación de alcaloides tropánicos (Hyoscyamine y escopolamina) afectaba a toda la planta (espontánea y cultivada) y a los diversos órganos y se llevó a cabo mediante el método colorimétrico y la cromatografía líquida de alto rendimiento (HPLC).

Resultados: El ensayo colorimétrico mostró que el nivel más alto de alcaloides se observó en la estación Adrar Sbaa (2,83 %) en el órgano foliar. Sin embargo, el órgano madre mostró un nivel promedio de alcaloides en todas las estaciones de recolección (de 0,5 a 0,98 %).

El ensayo de CLAR confirmó la riqueza de alcaloides e hiosciaminas en todas las estaciones de estudio para plantas espontáneas y especies cultivadas. La planta de las dos estaciones (Tamanrasset y Adrar Sbaa) se destacó con grados que alcanzaron (6,693±0,555 mg/100gDM y 4,707±0,092 mg/100gDM) respectivamente, y una tasa de hyoscyamine de (5,765± 0, 23 mg/ 100gDM) para la estación Tamanrasset.

Conclusiones: Al final de nuestro estudio y del contenido de los resultados obtenidos en *Hyoscyamus muticus* subsp *falezlez* (Coss.) Maire de Argelia, es imperativo explotar esta especie como fuente industrial de producción de atropina en Argelia.

Palabras clave: Hyoscyamine; Algeria; *Hyoscyamus*; HPLC.

Abstract

Introduction: *Hyoscyamus muticus* L. subsp *falezlez* (Coss.) Maire is a Saharan species rich in tropane alkaloids (especially hyoscyamine). Hyoscyamine is raced into atropine, whose interest in pharmacy is considerable.

The objective is to dose *Hyoscyamus muticus* L. subsp *falezlez* (Coss.) Maire alkaloids from the stations of Algerian Sahara (Abadla, Adrar, and Tamanrasset), to exploit the data in the valorization of this species as a potential source of industrial production of atropine.

Method: The determination of tropane alkaloids (Hyoscyamine and scopolamine) concerned the whole plant (spontaneous and cultivated) and the various organs and was carried out by colorimetric method and High-performance liquid chromatography (HPLC).

Results: The colorimetric assay showed that the highest level of alkaloids was observed in the Adrar Sbaa station (2.83 %) in the leaf organ. However, the stem organ showed an average level of alkaloids in all harvesting stations (from 0.5 to 0.98 %).

The HPLC assay confirmed the alkaloid and hyoscyamine richness in all study stations for spontaneous plant and cultivated species. The plant of the two stations (Tamanrasset and Adrar Sbaa) stood out with grades reaching (6.693**±**0.555 mg/100gDM and 4.707±0.092 mg/100gDM) respectively, and a hyoscyamine rate of (5.765± 0.23 mg/100gDM) for the Tamanrasset station.

Conclusions: At the end of our study and the content of the results obtained on *Hyoscyamus muticus* subsp *falezlez* (Coss.) Maire of Algeria, it is imperative to exploit this species as an industrial source of atropine production in Algeria.

Keywords: Hyoscyamine; Algeria; *Hyoscyamus*; HPLC

Highlight

Tropane alkaloids are essential drugs according to WHO data, they are always obtained from plants because their synthesis is expensive. *Hyoscyamus muticus* spp, are among the richest species.

No studies on the content of tropane alkaloids were carried out on Saharan Henbane from Algeria.

The results obtained make it possible to open the exploitation possibilities of the species studied given its richness in tropane alkaloids.

Introduction

Plants have always been a common source of medication, in the form of traditional preparations or pure active ingredients [1]. Alkaloids are the most important group of secondary metabolites of plants, both for their structural diversity and various pharmacological properties. Atropine and scopolamine are the most interesting molecules among the alkaloids of plants belonging to the Solanaceae family[2,3]. They are responsible for the development and introduction of more than 50 drugs[4] and are the starting point for the synthesis of most anticholinergic substances[5].

Hyoscyamus muticus L. subsp *falezlez* (Coss.) Maire is a widely distributed Solanaceae that grows spontaneously in the Algerian Sahara. It is part of the traditional medicine of the Tuareg. It contains tropane alkaloids predominantly represented by (Hyoscyamine and scopolamine). Hyoscyamine is raced into atropine during the extraction, it is this molecule that interests the pharmaceutical industry[6,7].

The dosage aims to highlight the richness of a plant in tropane alkaloids; several studies have treated the determination of the contents of these two tropane alkaloids with different analytical techniques (CPG/SM after transformation into trimethylated derivatives, liquid chromatography on reverse phase, capillary electrophoresis, immunological methods.)[8]. Besides biotechnological methods development, high-performance liquid chromatography (HPLC) is commonly used[9]. The volumetric colorimetric technique despite its basic character is still relevant because it is the technique of the European Pharmacopoeia 10th edition.

The levels of alkaloids of *Atropa belladonna* are of the order of (0.3 %), those of *Datura stramonium* (0.25 %)[10], the Egyptian Henbane exceeds 1 %, reaching 5 % for cultivated species, however, some Henbane of Iran do not seem to be very rich (0.027 %) or even black Henbane, which barely reaches 0.05 %[4,8].

It has been reported that the alkaloid yield expressed in hyoscyamine of Egyptian Hanbane of leaves, stems, flowers, and fruits were respectively: 1.39 %, 0.57 %, 1.34 %[11,12], so what about *Hyoscyamus muticus* subsp *falezlez* (Coss.) Mayor of Algeria? No study in Algeria has treated it.

In this study, we are interested in the determination of the two alkaloids content (Hyoscyamine and scopolamine), in the Saharan Henbane of Algeria. It allows us to know the place of this species among the other species with tropane alkaloids and what the potentialities that it can present to be a source of atropine for the pharmaceutical industry as well as the Henbane of Egypt.

The chemical analysis concerned the extraction residues of *Hyoscyamus muticus* L. subsp *falezlez* (Coss.) Maire that grows spontaneously and comes from three harvesting areas of the Algerian Sahara (Abadla, Adrar, Tamanrasset). It also concerned the species cultivated in the Adrar Sbaa region.

Methods

Plant material

The spontaneous plant was collected in June 2020, in the harvesting areas listed in Table 1 *Hyoscyamus muticus* L. *falezlez* (Coss.) Maire was harvested no more than three weeks after flowering for better alkaloid yield.

Table 1. *Hyoscyamus muticus* L. subsp f*alezlez* (Coss.) Maire harvest areas for chemical analysis

Harvesting areas	Stations	GPS coordinates
ABADLA (BECHAR)	Abadla	31.281, -2.439
ADRAR	Sbaa	28.205, -0.172
	Zawiet Kounta	27.253, -0.204
TAMANRASSET	In Ekker	24.016, 5. 081

For each station, thirty spontaneous plants were collected. Plant material was sorted (different parts), dried in the open air, and in the dark in a dry and ventilated place for three weeks. Then, they kept Kraft paper bags away from moisture. Plants were reduced, into a fine powder just before extraction.

The harvest of the cultivated plant was carried out in June 2022 from the station Adrar Sbaa, where the trial of cultivation by sowing.

Nonplant material

All reagents, chemicals, and controls used were of analytical quality and were supplied to us from the Pharmacognosy laboratory (Tlemcen) and the pharmacology department (University Hospital of Oran). They are represented by: a continuous flow extractor type Soxhlet, and high-performance liquid chromatography coupled with a UV detector (HPLC-UV).

Extraction of tropane alkaloids

The extraction of alkaloids is based on the technique of the European Pharmacopoeia 10th edition.

The first step is the weighing of each sample; it was carried out from a test sample of 10 g of the whole plant from the three harvest areas Abadla (ABD), Adrar (Adrar Sbaa, ADS, Adrar Zaouiet Kounta, ADZ), and Tamanrasset (TAM).

The solubility of alkaloids and their forms depend closely on pH variations, a feature that allows them to be separated from other plant constituents. In an acidic medium, they are in the combined form of «alkaloid salts», bound to organic acids at the plant level in this form they are soluble in aqueous solutions, and in a basic medium, they are in the free state and are therefore soluble in organic solvents[4].

The extraction followed the following steps:

• Alkaloids moved from their combination by a base: The powder from each sample was introduced into a beaker and then moistened with ammonia (NH_4OH 5 %) while homogenizing.

• Extraction by nonpolar organic solvent: The moistened powder is then transferred to a filter paper cartridge and fed into the intermediate part of the Soxhlet.

Enough dichloromethane (CH_2Cl_2) is introduced into the flask. Extraction is conducted for three hours with a temperature of 40°. The organic extract of alkaloids (bases) is obtained at the end. The resulting organic extract is introduced into a decanting ampoule, and then extraction is carried out by sulfuric acid (H_2SO_4 0.5 N); 15 mL for each extraction, the operation is repeated until the Mayer reagent test is negative (testifying to the absence of alkaloids).

• Purification: It consists of passing the extractive solutions, in an acid medium or a basic medium to eliminate water-soluble impurities and fat-soluble impurities[5]. The aqueous phases of extractions are collected and the resulting solution (containing alkaloids as salts) is alkalized by NH_4OH 5 % until the pH is between 10-12.

The alkaline solution is transferred to the decanting ampoule, and extractions with the 15 mL dichloromethane were made as many times as with H_2SO_4 0.5 N. The organic phases resulting from the extraction (which contain the alkaloids in the basic state) are joined then a wash with distilled water (10 mL) is carried out. The water is eliminated by decantation, and the organic phase is dried on anhydrous sodium sulfate (Na_2SO_4), which is eliminated by filtration in a dry round bottom flask.

The organic phase is evaporated using a rotavapor to obtain the dry residue of alkaloid bases.

The extraction steps are summarized in the following diagram, in Fig 1:

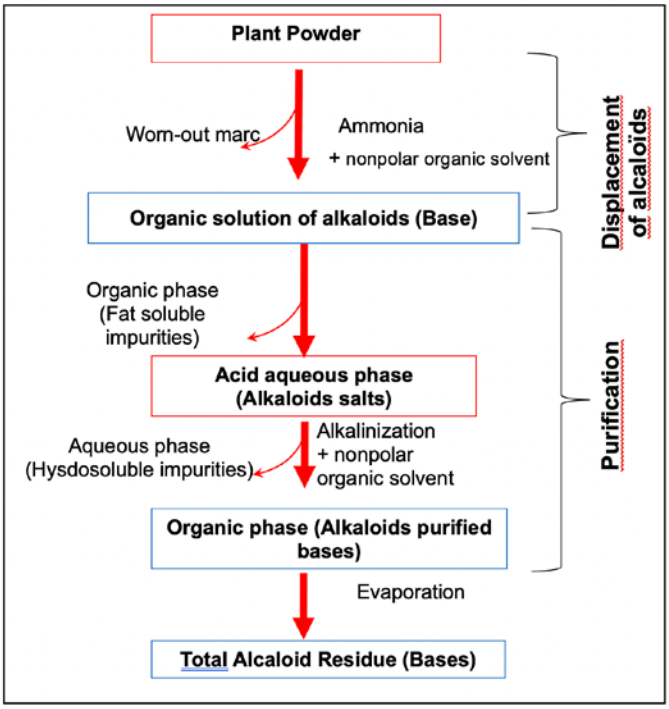

Figure 1. Diagram of alkaloid extraction

Colorimetric determination of total alkaloids in different parts of the plant in the three harvest areas

The weights of the test catches of the different plant organs of the harvest areas, used for the colorimetric determination are shown in the table 2 below.

Table 2. Test catch masses for total alkaloid extraction from each part of the three harvest areas.

Code	Organs	Mass taken test (g)
AB-005	Stems	1.69
AB-006	Leaves	3.29
AB-007	Flowers	3.91
AB-008	Seeds	0.53
ADS-013	Stems	1.89
ADS-014	Leaves	2.56
ADS-015	Flowers	3.72
ADS-016	Seeds	1.4

Code	Organs	Mass taken test (g)
ADZ-021	Stems	1.9
ADZ-022	Leaves	2.09
ADZ-023	Flowers	2.62
ADZ-024	Seeds	2.17
TA-029	Stems	2.85
TA-030	Leaves	4.35
TA-031	Flowers	4.24
TA-032	Seeds	3.98

AB: Abadla Station, ADS: Adrar Sbaa, ADZ: Adrar Zaouiet kounta, TA: Tamanrasset

The dry residue of total alkaloids extraction from each organ was solubilized in 20 mL of anhydrous acetic acid, and a drop of methyl violet was added to the flask. A 25 mL can be filled with perchloric acid $HClO_4$ 0.01 N[13]. The principle of colorimetric determination is to assess the level of alkaloids neutralized by an acid; thus the alkaloid level is proportional to the number of mL of perchloric acid needed to displace the alkaloids from their combination resulting in the shift from green to purple[13].

A calibration curve previously established by the pharmacochemistry laboratory of the Faculty of Pharmacy of Valencia allowed us to know the mass of hyoscyamine neutralized by 1 mL of perchloric acid ($HClO_4$ 0.01 N). Thus 1 mL of this acid would correspond to 0.00289 g of alkaloids expressed in hyoscyamine. The quantity found is per 100 g of dry matter[13]. A rule of three allowed us to deduce the amount of alkaloids from the mass of the test taken for each part of the plant.

N: Number of mL of perchloric acid required to neutralize alkaloids contained in the extract.

A: alkaloid yield per 100g of dry drug

P: Mass taken test (g)

Determination of whole alkaloids in spontaneous and grown plants, by high-performance liquid chromatography (HPLC)

The determination of the tropane alkaloid content in our extracts was carried out by an optimized method[8,14,15].

Chromatographic analysis using high-performance liquid chromatography, was performed for:

- Extraction residues from whole plants from the three harvest areas (Abadla, Adrar (Sbaa and Zaouiet Kounta) and Tamanrasset);
- Extraction of residues (by reflux from whole plants grown in the Adrar Sbaa area).

Operating conditions

A uHPLC with automatic injection, connected to a UV-visible detector (190-600nm), was used. It consists of a Perkin-Elmer-USA pump, a binary gradient Chrom 7100, an automatic injection valve, and a Perkin-Elmer-USA LCI-100 integrator.

The analysis was done in an isocratic mode. The flow rate was 1 mL/min, and the column used is of type C18 of 25 mm length and 4 mm diameter. The injection volume was 20 µL and the detection wavelength λ=210 nm.

Composition of the mobile phase

It is composed of 85 % phosphate buffer (25 mM) pH=7 and 15 % acetonitrile grade HPLC according to the protocol[14].

Preparation of phosphate buffer solution at pH 7

The preparation of the 25 mM phosphate buffer was carried out by referring to the European Pharmacopoeia 10th edition as follows:

To obtain a 25 mM phosphate buffer it is necessary to prepare a 63 mM phosphate buffer by mixing two salts, Na_2HPO_4 with NaH_2PO_4: 5.18 g of Na_2HPO_4 was mixed with 3.65 g NaH_2PO_4; both salts were introduced into a 1 L cylinder and 950 mL of distilled water was added. The pH was adjusted with phosphoric acid until it reached an interval of (4.5-6.2), then the solution was supplemented at 1 L with distilled water.

A volume of buffer solution (63 mM) was mixed with 1.5 volumes of distilled water to obtain a buffer solution at pH 7 (25 mM).

The two solvents of the mobile phase (phosphate buffer and acetonitrile) were mixed, filtered, and then degassed by ultrasound for 5 minutes.

Standards were Hyoscyamine sulfate (Pharmacopeia reference Standard, Id: 006XO1) and Scopolamine bromhydrate (Sigma –Aldrich, PHR1470).

Each control was diluted in 1 mL ethanol (HPLC grade). The resulting solution was mixed, centrifuged for 10 min, and filtered on a microfilter before injection.

Solutions to be analyzed

The extraction residue was diluted in 1 mL ethanol (HPLC grade). The resulting solution was mixed, centrifuged for 10 min, and filtered on a microfilter before injection. For all samples, the analysis was repeated twice.

Preparation of the standard range

The standards were weighed using an Eppendorf tube on an analytical scale (0.001g precision). 1 mg of Hyoscyamine sulfate and Scopolamine were solubilized in 1 mL of ethanol (HPLC grade)[14].

Five dilutions were prepared from the initial solutions of each standard at different concentrations with a dilution factor of ½. Dilution was performed in ethanol. The solutions were mixed, centrifuged, and filtered for 10 minutes before injection.

Calculation of total alkaloid content of samples after HPLC analysis

The different concentrations of the standards (Hyoscyamine sulfate and Scopolamine bromhydrate) allowed us to draw the two corresponding calibration curves from the areas under the curve of the two alkaloids and their dilutions (see Fig 2, Fig 3). The linear regression function linking the area under the curve of each standard and its alkaloid concentration (see Table 3) was derived from the calibration curves drawn. Each analysis was repeated twice.

Table 3. Linear regression equation and correlation coefficient of Hyoscyamine and Scopolamine (n=2)

Standards	Linear regression equation	correlation coefficient (R2)	LOD (mg/L)	LOQ (mg/L)
Hyoscyamine	Y=555.91X-49.218	0.9985	0.5	1
Scopolamine	Y=309.18X- 26.885	0.9997	0.5	1

Y: area under the curve, X: alkaloid concentration (mg/mL), LOD: Detection limit, LOQ: Quantification limit, n: number of replicates

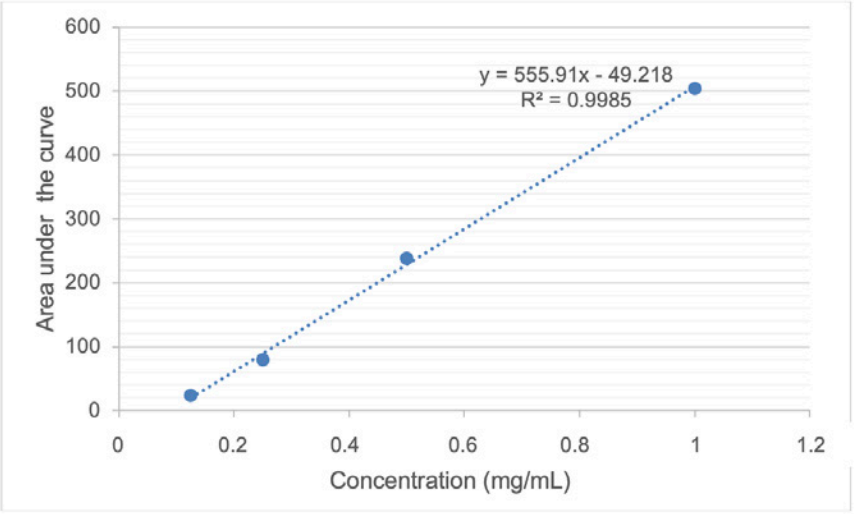

Figure 2. Hyoscyamine calibration curve

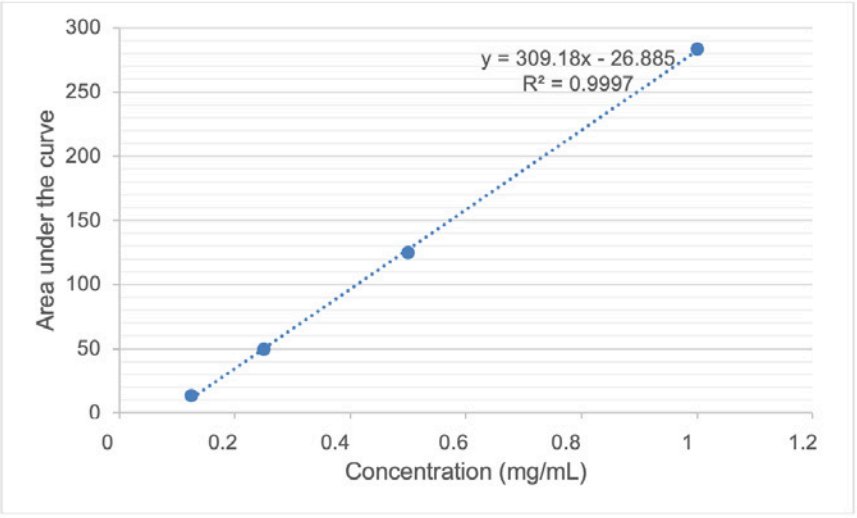

Figure 3. Scopolamine calibration curve

The concentrations of the two alkaloids (Hyoscyamine and Scopolamine) from the extracts of the different stations were calculated from the regression functions of the two calibration curves of each alkaloid.

The alkaloid (mg/100gDM) concentrations (Hyoscyamine and Scopolamine) of the analysed extracts were calculated using the amount of dry matter (DM) of each extraction.

The total alkaloid content in mg per 100g of dry matter is given by the formula below[16].

$$Total\ alcaloid\ yield\ (\frac{mg}{100gDM}) = (100a + 69.2b)/p$$

a and b are the concentrations of hyoscyamine and scopolamine (mg/mL), and p is the sample weight in grams. DM: Dry matter

Results

The results correspond to a chemical analysis of *Hyoscyamus muticus* L. subsp *falezlez* (Coss) Maire by:

- Colorimetric method of plant organs in the harvesting station,
- HPLC of the alkaloids of the whole spontaneous plant from the three harvest areas, and those of the plant grown in the Adrar Sbaa station.

Colorimetric determination of total alkaloids in different parts of the plant in the three harvest areas

Alkaloids are concentrated in the leaves in the three stations (Adrar Sbaa 2.83 %, Abadla 2.45 % and Adrar Zaouiet kounta 0.98 %) followed by flowers (with 1.52 % for the Abadla station) and seeds. However, for the Tamanrasset station, the highest level of alkaloids was observed in the seeds (0.61 %) at this time of year.

The highest level of alkaloids was observed in the Adrar Sbaa station (2.83 %) in the leaf organ.

The stem organ showed an average alkaloid level in all stations for this harvest period (from 0.5 to 0.98 %). The table below (see Table 4) summarizes the results of the colorimetric determination of total alkaloids, performed on the different parts of *Hyoscyamus muticus* L. *falezlez* (Coss.) Maire, from the different harvesting stations.

Table 4. Percentage of total alkaloids by organ

Organ	Stations			
	Total alkaloid yield (%)			
	ABD	ADS	ADZ	TAM
Stem	0.83	0.943	0.41	0.5
Leaves	2.45	2.83	0.98	0.53
Flowers	1.52	1.01	0.5	0.4
Seeds	1.14	1.4	0.53	0.61

AB : Station Abadla, ADS : Adrar Sbaa, ADZ : Adrar Zaouiet kounta, TA : Tamanrasset

Results of HPLC analysis of alkaloids from spontaneous plant

The HPLC analysis of the standards (Hyoscyamine and Scopolamine) showed a peak at 6.5th minute corresponding to the scopolamine and a peak at 9.8th minute corresponding to the hyoscyamine, the spectral data of the two standards taken as examples (Hyoscyamine concentration 1 mg/mL and scopolamine concentration 0.125 mg/mL are summarized in Figures 4 and Figure 5).

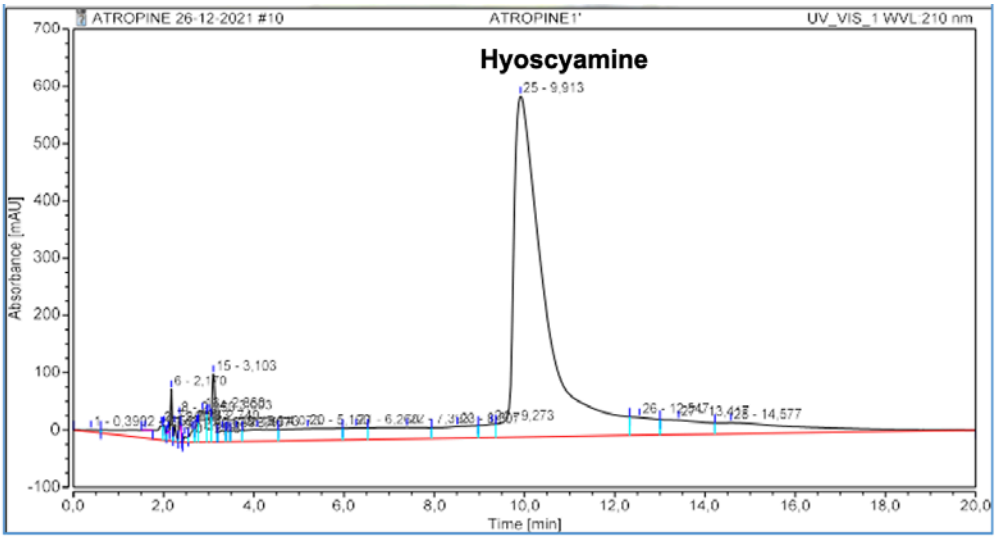

Figure 4. Hyoscyamine standard chromatogram, 1 mg/mL concentration

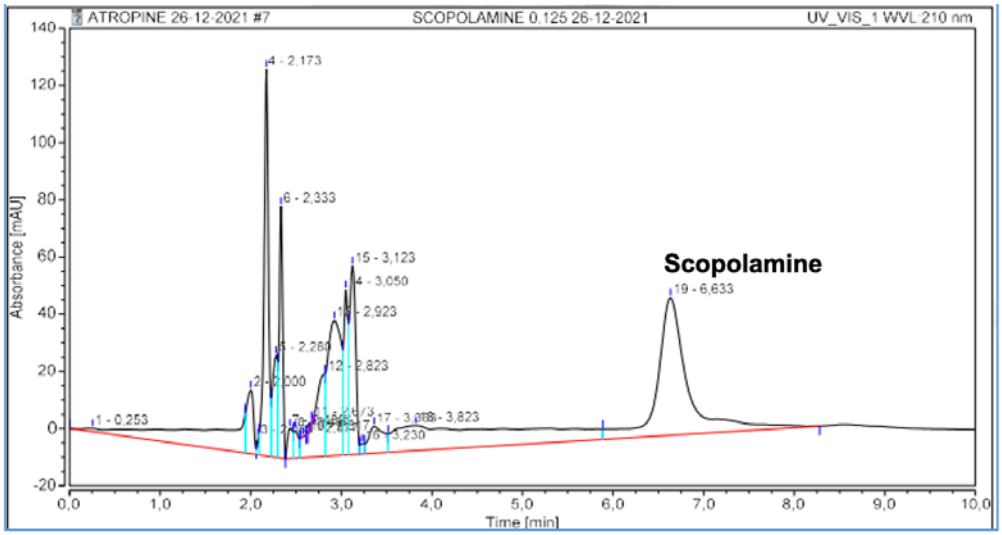

Figure 5. Standard scopolamine chromatogram, 0.125 mg/mL

The UV chromatogram of the identified alkaloids showed two detectable peaks corresponding to a significant hyoscyamine peak with a retention time of 9.8 min, and a percentage ranging from (80 to 95 %) and a minor scopolamine peak (retention time of 6.5 min, with a percentage ranging from 1 to 8 %.). The Fig 6 concerned Abadla station chromatogram.

Other minor alkaloids were detected after the 10th minute that may correspond to tropic acid and apotropic acid based on studies using a similar protocol[16,17].

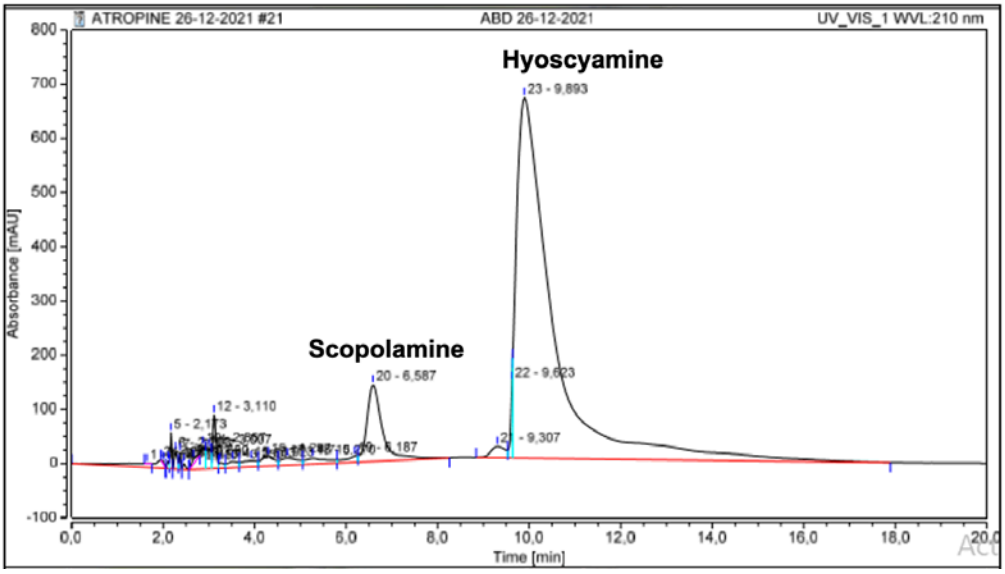

Figure 6. Chromatogram of atropine and scopolamine assay by HPLC (Abadla station)

Hyoscyamine and scopolamine contents in plant extracts from the three harvest areas expressed in (mg/mL) were obtained from linear regression functions of the calibration curves of the two standards (Hyoscyamine and scopolamine).The spectral data of the harvest areas are summarized in Table 5.

Table 5. Concentration (mg/mL) and percentage of alkaloids (Hyoscyamine and scopolamine) dosed by HPLC according to harvest areas, n=2

Harvest área		Hyoscyamine (mg/mL)	Hyoscyamine (mg/100gDM)	Scopolamine (mg/mL)	Scopolamine (mg/100gDM)	Total alkaloid mg/100gDM
Abadla (ABD) 2.848		2.829	2.931	0.604	0.229	3.246
		2.956	0.601	0.306	3.263	
Adrar	ADS	3.979	4.571	0.175	0.05	4.8
		3.749	4.153	0.178	0.057	4.614
	ADZ	2.611	2.625	0.156	0.04	2.718
		2.653	2.597	0.157	0.039	2.761
Tamanrasset (TAM) 6.627		5.635	5.527	0.727	0.354	6.138
		6.003	0.90	0.417	7.249	

n: number of tests

The percentage of hyoscyamine between stations varies between 82.82 % and 96.59 % while that of scopolamine oscillates between 1.2 % and 9.4 %; a variation that occurred although the plants were harvested during the same period.

The Tamanrasset (TAM) and Adrar Sbaa (ADS) stations yielded significant yields of total alkaloids (6.693±0.555 mg/100gDM and 4.707±0.092 mg/100gDM respectively), followed by the Abadla (ABD) station which showed a relatively high rate (3.254± 0.008 mg/100gDM). The station of Adrar zaouiet

kounta (ADZ) showed a low rate compared to the others especially compared to the station of Adrar Sbaa (2.739± 0.021 mg/100gDM) despite their geographical proximity.

As for the hyoscyamine and scopolamine contents, the Tamanrasset station stood out with a high hyoscyamine rate compared to other stations (5.765± 0.23 mg/100gDM). Abadla and Adrar Zaouiet Kounta had similar hyoscyamine levels (2.943± 0.012 mg/100gDM), (2.61± 0.014 mg/100gDM).

For scopolamine, rates range from 0.2 to 0.3 mg/100gDM for all stations.

The Table 6 summarizes the mean concentrations of hyoscyamine and scopolamine (mg/100gDM) and total alkaloids of plant extracts from the three harvest areas.

Table 6. Hyoscyamine, scopolamine, and total alkaloids concentration (mg/100gDM) averages, HPLC assays based on harvest areas.

Harvest area	ABD	ADS	ADZ	TAM
Hyoscyamine (mg/100gDM)	2.94±0.012	4.36±0.209	2.61±0.014	5.76±0.23
Scopolamine (mg/100gDM)	0.26±0.038	0.05±0.003	0.03±0.005	0.38±0.031
Alkaloid Yield (mg/100gDM)	3.25± 0.008	4.70±0.092	2.73±0.021	6.69±0.555

DM: Dry Matter

Results of the HPLC analysis of alkaloids of the whole cultivated plant

Tables 7 report the alkaloid levels of the plant from the Adrar Sbaa farm culture test, after reflux extraction and purification. There is a high content of total alkaloids (4.104±0.419 mg/gDM) and hyoscyamine (3.7±0.35 mg/gDM).

Table 7. Alkaloids (hyoscyamine and scopolamine) concentration (mg/mL) and percentages by HPLC of the cultivated plant a Adrar Sbaa, n=2

Harvest area	Hyoscyamine concentration (mg/mL)	Hyoscyamine concentration (mg/100gDM)	Scopolamine concentration (mg/mL)	Scopolamine concentration (mg/100gDM)	Alcaloïd mg/100g DM
Adrar Sbaa	4.44	4.06	0.12	0 .13	4.52
	3.61	3.35	0.13	0 .07	3.69

Discussion

The yield of total alkaloids in the different parts of the plant was high for the leaf; 2.83 % was obtained for the station of Adrar Sbaa and 2.45 % for the flowers. The maximum rate was observed in the station Abadla, 1.52 %.

The results found join the classification of the organs of the Egyptian species except for the flower according to two studies[11,12], the alkaloids of the leaves represent 1.7 %, those of ripe fruits 1.34 %, stems 0.569 % and flowers 2 % the levels obtained for the leaves of our Henbane are higher. In Turkey, a study concerned a neighbouring species with tropane alkaloids, *Hyoscyamus reticulatus*, its levels were lower than those of our species except for the stem where it displays 0.8 %, however for the cultivated species alkaloid levels for all organs exceed that of *Hyoscyamus muticus* L. *falezlez* (Coss.) Maire from Algeria[18].

Another study in Iran that involved three related species including *Hyoscyamus reticulatus* reported lower rates than those obtained by our species except for seeds where they were comparable[8].

The HPLC analysis of the extracts revealed two majority alkaloids, comparing their retention time with those of the standards; hyoscyamine was detected around the 9th minute, and scopolamine, was detected around the 6.5th minute. Other minor alkaloids were detected after the 10th minute, corresponding to tropic and apotropic acid. Not having the standards of these two compounds, we referred to a study relating to the qualitative and quantitative analysis of the alkaloids of three Solanaceae including the Black Henbane[16]. In all the chromatograms of the study, the two compounds (tropic and apotropic acids) came out after the 10th minute.

The assessment of total tropane alkaloids by HPLC of the collected spontaneous plant samples showed that the Tamanrasset (TAM) and Adrar Sbaa (ADS) stations gave significant yields of total alkaloids (6.693± 0.555 mg/100gDM and 4.707± 0.092 mg/100gDM respectively) followed by the Abadla station (ABD) which showed a relatively high rate. The station of Adrar Zaouiet kounta (ADZ) showed a low rate compared to the others, especially the station of Adrar Sbaa despite their geographical rapprochement, but its rate remains high. The difference in alkaloid and especially hyoscyamine levels between the different stations, can be explained by geographical location and climatic conditions of each region. This partly justifies the comparison of the plant content and richness of the Tamanrasset station with that of the plant studied by another investigator in the Ahaggar area[3]. Another factor that may explain this difference and may influence this variability in levels, is the irregularity of the vegetative stage of the harvested plants. Although they were harvested in the same period, the plants showed differences in the development of their vegetative apparatus; the plants at the Tamanrasset and Adrar Sbaa stations were still green while those at the Abadla station had begun to dry out, except for a maximum of alkaloids the harvest must be done 3-4 weeks after flowering[12].

Also, irrigation is fundamental for the survival of the plant; the plant needs water during the first days and when it reaches 60cm deep. Irrigation should not be too conditional because the water allows it to have a developed vegetative apparatus but does not depend on the concentration of alkaloids[12]. All these factors do not allow us to draw a chemical profile of the species studied for each region without studying a larger sample with many stations for each region, however, it informed us about the potential of the two stations (Adrar Sbaa and Tamanrasset).

The maximum total alkaloid content obtained was 6.69 with the Tamanrasset station, the result joining the study made from samples taken from the Ahaggar region which showed a total alkaloid level of 4.88 mg/100gDM according to the Boukhalfa study in 2017[3]. The results also agree with the results of a study in Egypt by Zolala J. in 2007, which reported levels exceeding 5 mg/100gDM[20].

Hyoscyamine concentrations in Algeria, according to a study in the Ahaggar region, are about 3.73 mg/100gDM[3], while the Tamanrasset station showed a rate of 5.76 mg/100gDM. This confirms the wealth of the Tamanrasset station.

Hyoscyamine contents of *Hyoscyamus muticus* L. *falezlez* (Coss.) Maire are higher than those of the *Hyoscyamus* genus, studied in Iran by Bahmanzadegan, J A in 2009 whose contents vary between 0.3 and 1.9 mg/100gDM[8].

The HPLC analysis of the plant grown at Adrar Sbaa gave a satisfactory content of total alkaloids (4.1 mg/100gDM) and hyoscyamine (3.7±0.35 mg/ 100gDM) which once again confirms the richness of *Hyoscyamus muticus* L. *falezlez* (Coss.) Maire; the levels are comparable to that of the spontaneous plant harvested from the same area.

However, the results obtained remain lower than those of the species cultivated in Egypt whose total alkaloid levels exceed 6 mg/100gDM according to the study of Zolala J. in 2007[20].It can be explained by the development of cultivation techniques in Egypt and soil enrichment, the improvement of tropane alkaloid-producing strains[19], and the exploitation of this species in Egypt for a very long time[21].

Hyoscyamus reticulatus was studied in Iran, could compete with Saharan henbane as it had the highest rate (5.8 mg/100gDM)[8], another study in Turkey by Al-Sanafi, in 2021 also confirms its wealth[18].

Conclusion

The study objective was to measure the alkaloids of *Hyoscyamus muticus* L. *falezlez* (Coss.) Maire of Algeria, to valorize it as an industrial source of atropine production. It is a Saharan species little studied in Algeria compared to *Datura* spp and other *Hyoscyamus* spp, encountered in the North of the country.

The analysis of its chemical profile, specifically the alkaloid part, allowed us to provide additional confirmation on its richness and pharmaceutical interest. The study of the different stations (Abadla, Adrar, and Tamanrasset) has never been the subject of a single study in Algeria, the results highlighted the variations of the grades in the studied stations and made it possible to identify those which can have promising cultural capacities (Adrar Sbaa and Tamanraset). The two stations stood out in chemical analysis, with concentrations reaching (6.693±0.555 mg/100gDM and 4.707±0.092 mg/100gDM, and a hyoscyamine rate of (5.765 ±0.23 mg/100gDM) for the Tamanrasset station.

The alkaloid content of the cultivated plant measured by HPLC was very satisfactory and compared with the spontaneous plant with a content of 4.104±0.419 mg/100gDM of total alkaloids and 3.7±0.35 mg/100gDM of Hyoscyamine.

The tropane alkaloid yields in our samples, both for the whole plant and the different organs were interesting and comparable to those of Egyptian henbane and the other species from Turkey and Iran. *Hyscocyamus muticus* L. subsp *falezlez* (Coss.) Maire of Algeria recorded total alkaloid contents of 2.83 % and 2.45 % for the leaves of the Adrar Sbaa and Abadla stations and reached 1.4 % for the seeds of Adrar Sbaa.

The results of the chemical analysis show that *Hyoscyamus muticus* L. *falezlez* (Coss.) Maire from Algeria can be an important source of atropine as much as Eypte's Henbane.

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