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Identification of Alkaloids in Methanol Extract of *Datura Stramonium* Plant from Iran

Alireza.Sardashti*¹, Tayebe. pourgharib Shahi¹

1. Department of Chemistry, Faculty of Science, University of Sistan and Baluchestan, P.O.Box 98135-167, Zahedan, Iran.

ABSTRACT

All parts of *Datura* plants contain dangerous levels of the tropane alkaloids atropine, hyoscyamine and scopolamine which are classified as deliriants, or anticholinergics. The aerial parts of *Datura stramonium* were harvested in flowering stage from botanic garden of Sistan and Baluchestan university in the mid, June 2012. The methanol extract was gathered by soxhlet apparatus and concentrated in rotary. We could generate total alkaloid solution by the use of sulfuric acid, ammonia and chloroform. The alkaloid total was purified by passing from a column of silica gel and was washed by a combination of ether petroleum and chloroform solutions. The existence of alkaloid and purity of the generated solution from the column by the thin-layer chromatography technique was confirmed. Then this solution was analyzed by GC-MS technique. The scopolamine alkaloid was identified with the amount of 5.77%. This combination is the reason of antioxidant feature of the plant extract.

Keywords: *Datura stramonium* plant; Methanol extract; GC-MS technique, Scopolamine alkaloid.

*Corresponding Author Email: sardashti@chem.usb.ac.ir

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INTRODUCTION

Datura stramonium L. is a one-year-old herbal plant with a height of 30 to 80 cm. This plant sometimes grows over one meter in height¹. *Datura stramonium* L. is an erect branched under shrub with long white flowers and spiny spherical fruits². This plant is distributed throughout most parts of temperate regions of the world. According to the recent classifications, it has four varieties, *D. stramonium* var. *stramonium* L., *D. stramonium* var. *tatula* L., Torr, *D. stramonium* var. *inermis* Jacq. Timmerman and *D. stramonium* var. *godronii* Danert, which had been considered for years by many botanists as different species^{3,4,5}. Among *Solanaceae* plants, *Datura stramonium* L. is highly regarded by the workers, since it has a great resource of tropane alkaloids⁶. Tropane alkaloids play an important role in medicinal and defensive industries⁷. Botanical alkaloids are one of the most important botanical products and form a major part of medicinal compounds⁸. Tropane alkaloids have a chemotaxonomical value for the *Solanaceae*⁹. To our knowledge; the typical variety *D. stramonium* L. var. *stramonium* has a well-established alkaloid pattern whereas only hyoscyamine and scopolamine have been reported for the remaining varieties^{10,11,12}. Tropane alkaloids (i.e. atropine, hyoscyamine, and scopolamine) are generally found in *Hyosyamos* and *Datura stramonium* L.¹³. Medicinal plants are of great importance to the health of individuals and communities. These plants are sources of inspiration for novel drug compounds. Modern scientists have made phenomenal steps in development of this heritage, which is handed over by our ancestors¹⁴. Plants usually contain physiologically active substances, which are technically referred to as drugs over the years; these drugs have been exploited as traditional medicine for the treatment of various ailments Afflicting man¹⁵. The drugs obtained from medicinal plants are termed crude drugs of natural or biological origin by pharmacists and pharmacy logists¹⁶. All parts of these plants are toxic, but the highest amount of the alkaloids is stored in ripe seeds¹⁷. Many cases of accidental poisoning by *D. stramonium* species have been reported when these plants were eaten accidentally or when decoction is prepared based on herbal prescriptions¹⁸ (Intentional poisoning with *D. stramonium* has also been reported in several cases, for example fatal poisoning with *D. stramonium* for its mind altering properties and *Datura* eating or chewing in a suicides attempts¹⁹⁻²³). The toxicity of *D. stramonium* in grazing animals have been suspected by livestock owners and field veterinarians especially at the time of drought or after ingesting freshly harvested maize that are used for ensiling and are heavily contaminated with young *D. stramonium*²⁴. The toxin of this plant that contains tropane alkaloids is regarded as a classic anti-cholinergic poison²¹⁻²⁵. Most reported cases of DS poisoning occurred among teenagers after they ingested it

voluntarily for its hallucinogenic and euphoric effects²⁶⁻²⁹. This report illustrates an unusual case of DS poisoning, occurred in a geophagous 3.5-year-old child after accidental ingestion of the plant's foliage with earth. Several analytical techniques have been employed for the analysis of hyoscyamine (atropine) and scopolamine. Moreover the enzyme-linked immunosorbent assay (ELISA)³⁰ and the spectrometric methods (based on indirect determination of alkaloid drugs by formation of ion-associate metal complexes of the drugs) like atomic emission spectrometry (AES)³¹, atomic absorption spectrometry (AAS)³¹, UV spectroscopy³¹, as well as various separation techniques are frequently applied. Capillary electrophoresis (CE) has been recommended as an inexpensive method for the study of pharmacological and plant samples. Several approaches have been published for capillary zone electrophoresis (CZE)³²⁻³⁸ and micellar electro kinetic chromatography–mass spectrometry (MEKC–MS)³⁹. Recently, CZE with MS interface³⁹, was proved to provide fast separation and high resolution. The disadvantage of common CE techniques is their relatively higher detection limit (LOD is around $5 \mu\text{g mL}^{-1}$)⁴⁰, however, coupling these techniques with an MS detector and using SIM mode can improve the detection limit by three to four orders of magnitude³⁸. Chromatographic methods have been represented by thin-layer chromatography (TLC)⁴¹⁻⁴³, high performance thin-layer chromatography–densitometry (HPTLC–densitometry)⁴³, gas chromatography (GC)³⁵, and gas chromatography–mass spectrometry (GC–MS),¹¹⁻⁴⁴ which were used for studying alkaloid patterns and/or quantities in various biological samples. Namera et al,⁴⁵ has identified The methanol extracts of the *Datura* plant alkaloids column chromatography and thin layer chromatography and GC-MS techniques.

MATERIALS AND METHOD

Plant material

Plant samples (*Datura stramonium*.L) were grown in the botanic garden of the university of Sistan and Baluchestan. The leaf and stem pieces were collected in flowering stage. Then, samples were dried at room temperature, kept in a dark place, and powdered. After that, the powder was passed through the sieve IR to produce uniform samples. Finally, the uniform samples were kept in a refrigerator at 4° C and a dark situation.

Alkaloid extraction

750mL of methanol was added to 80g of the powdered sample in a flask. Then, soxhlet apparatus for extraction of extract was performed for 12 hours. The extract was concentrated using rotary evaporator at room temperature and was mixed with 300mL sulfuric acid solution (2%) to form sodium acetate which is alkaloid after that, ammonia was added simultaneously and in a drop wise

manner to the solution until the pH level reached to 8.0. This process had three stages and in each stage, 50mL of chloroform was added to the resulting solution and was shaken for 20- 30 minutes. The organic phase was separated and concentrated by rotary ⁴⁶.

Column chromatography

Extract ant was separated on a silica gel column (SRL, 230-400 mesh). The column was washed with mixture of methanol – chloroform whose polarity was permanently incremented from 0:100 to 100:0. The 20 parts received were tested by TLC and GC-MS.

Thin layer chromatography

TLC was applied on all 20 parts but just 45:55 fraction answers were desirable. For this purpose silica gel page (with dimensions 30*30 cm) was placed in solvent tank Methanol-hexane, chloroform (4:1:5)ml for 5 minutes. Dragen-dorff reagents were used for detection of alkaloids.

Gas chromatography/mass spectrometry (GC-MS)

The GC-MS analysis was carried out on Agilent 6890/MSD5975B instrument operating in electron impact (EI) ionization mode at 70 eV, with MS transfer line temperature: 280 °C, ion source temperature: 230 °C, quadruple temperature: 150 °C, and mass range: 30–500 amu. An HP-5MS column (30 m×0.25 mm×0.25 µm) was used. The flow rate of the carrier gas (He) was 1 mL/min. The temperature program was 50 to 250 °C, ramped at 10 °C/min and held at the final temperature for 20 min. Injector temperature was 270 °C. The injection was performed in the splitless mode and the injected volume was 1 µL. The identities of the alkaloids were confirmed by comparing the measured mass spectral data with those obtained from the literature and database NIST ⁴⁷.

RESULTS AND DISCUSSION

Plant samples (*Datura stramonium*.L) were grown in the botanic garden of the university of Sistan and Baluchestan. After primary preparation, the samples were extracted with methanol by soxhlet. Raw alkaloids extract solution by sulfuric acid, ammonia and chloroform extraction kit. A UV-Vis spectrum of the sample extract of the flowering period was shown in Figure 1 and a peak at 255 nm was observed that indicated the possible presence of alkaloids. IR spectrum was shown in Figure 2. The IR spectrum peaks at positions 1729 cm^{-1} & 3421 cm^{-1} and observed that the order of the group NH, OH and the carbonyl (Figure 2). Identify compounds after column chromatography of the solution components in raw alkaloid extracted from the plant extract of *D.Stramonium*.L. for purification of the crude alkaloid solution, it was passed through silica gel chromatography column; column was washed with chloroform and methanol to separate the compound of sample. Solution obtained following column chromatography was analyzed by TLC

technique that confirmed the purification(Figure 3).TLC was applied on the eluent to confirm purification in the present study a GC and GC-MS procedure was applied for the identification of *scopolamine* alkaloids in the plant organs (stems and leaves) of *Datura stramonium* .L(Figures 4 and 5).

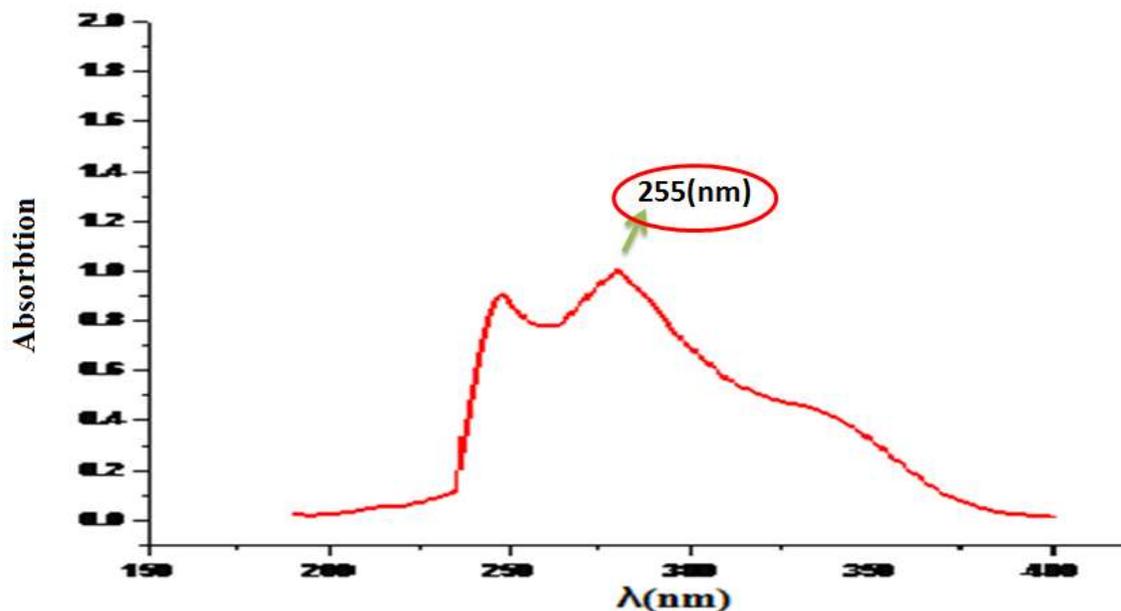


Figure 1: UV-VIS spectra of the raw alkaloid sample solution during the flowering *Datura* plant

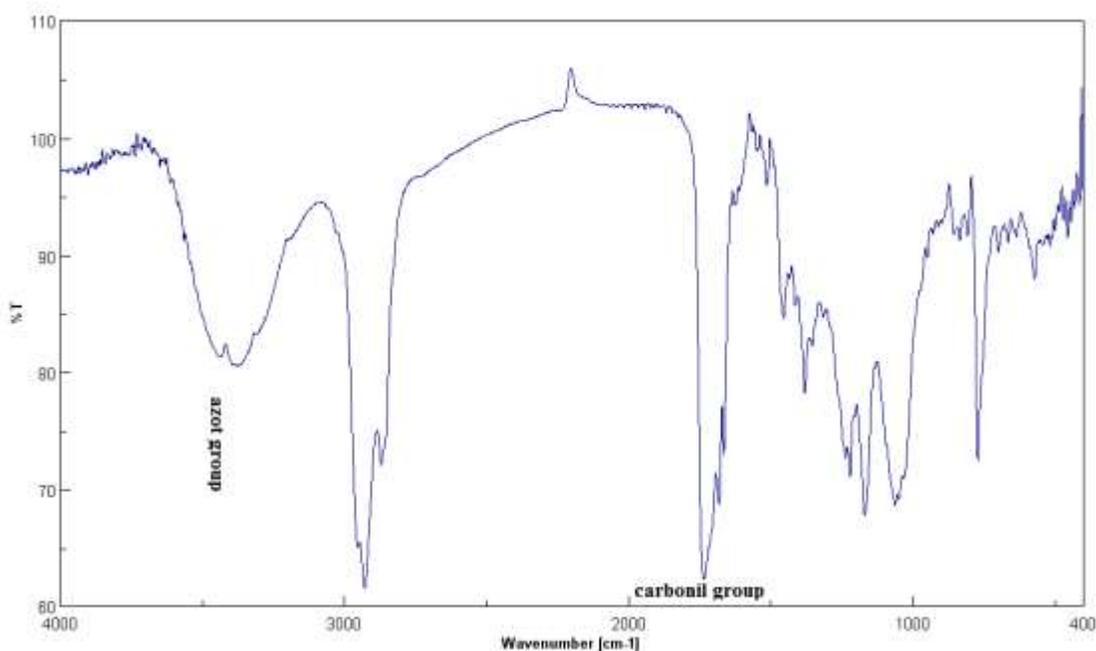


Figure 2: IR specturm of the raw alkaloid sample solution during the flowering plant *D.stramonium*.



Figure 3. TLC chromatogram of scopolamine alkaloid (45:55 fraction) solvent in tank

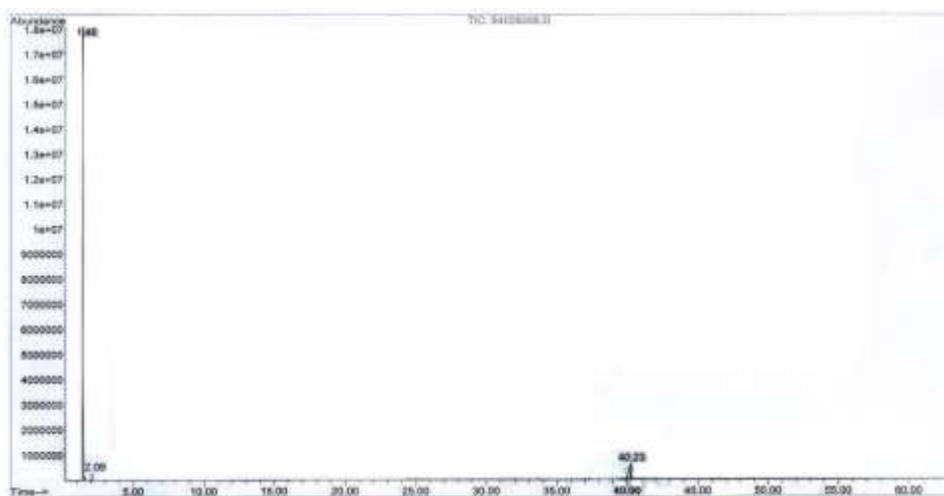


Figure 4. GC chromatogram of the sample solution from the column after elution by solvent

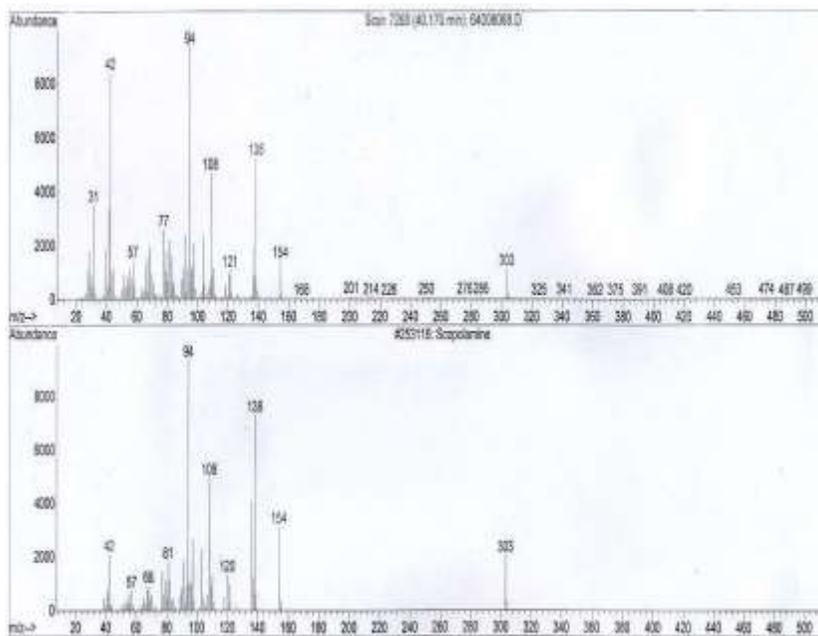


Figure 5. The mass spectrum (upper part: experimental, down: standard) of scopolamine alkaloid

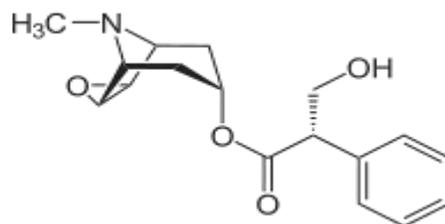


Figure 6. Structure of scopolamine alkaloid

Table 1: Interpretation of result is in agreement with a previous report

Plant	Compound	Identification method	references
<i>Datura stramonium</i>	3,7-dihydroxy-6-propionyloxytropane, 6,7-dehydro-3-tigloyloxytropane, 3-tigloyloxy-6,7-epoxytropane, 3,7-dihydroxy-6-(2'-methylbutyryloxy)tropane, 6,7-dehydroapoptropine, 3-(3'-methoxytropoyloxy)tropane, 3-tigloyloxy-6-isobutyryloxy-7-hydroxytropane, 3-tropoyloxy-6-isobutyryloxytropane, 3 β -tropoyloxy-6 β -isovaleryloxytropane	GC-MS	Laboratoire de Recherches et d'Analyses Techniques et Scientifiques de la Gendarmerie Royale (LARATES), Rabat, Maroc. BP: 6597 Rabat <i>instituts Maroc</i> ; Laboratoire de Génétique et Biométrie, Département de Biologie, Faculté des Sciences, Université Ib
<i>Datura stramonium</i>	Sixty-four tropane	GC-MS	Strahil Berkov a , Tsvetelina Doncheva, Stefan Philipov, Kiril Alexandrov ^{11,47}
<i>Datura stramonium</i>	Twenty-nine tropane	GC-MS	Stefan Philipov and Strahil Berkov ¹²
<i>Datura ceratocaula</i>	Forty Alkaloid(<i>scopolamine</i>)	GC-MS	Strahil Berkov ⁴⁸

Figure 4 .Showed one peak at retention time of 40.23 minutes for alkaloid .*Scopolamine* alkaloid appeared as one peak in GC-MS spectrum with identical mass Spectra. The stereochemistry of this alkaloid could not be established solely by MS data. It was suggested on the basis of their retention data reported in the literature (Figure 5).CG –MS spectrum showed the peak M⁺ (base ion) at m/z 303 corresponding to a molecular formula C₁₇ H₂₁ NO₄ indicated the presence of scopolamine and its value is equal to 5.77 % (Figures 4 and 5). TLC chromatogram of eluent of column chromatography, confirmed the presence of the alkaloid scopolamine (Figures 3 and 6).

Based on comparison with other paper

This result is in agreement with a previous report Berkov and Philipo 2003 .The highest scopolamine from *D.ceratocaulaplant* (Table1) content was found in the flowers(0.34 mg/g DW)⁴⁸.

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