DETECTION OF THE CONTENT OF SOME TOXIC AND NARCOTIC ALKALOIDS IN: LYCIUM DESERTI PHIL AND HYOSYAMUS ALBUS

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Abstract - Atropine, Nicotine and Hyoscine are Alkaloids produced by plants from the family Solanaceae as a secondary metabolite and which have an intense physiological action on animals even at low doses. The main source of Atropine, and Hyoscine alkaloids is the Datura spp wile the main source of Nicotine are Nicotiana spp. This study focuses on detecting the presence of Hyoscine, Atropine and Nicotine in other plant species from the family solanceae other than their known sources, namely the Hyosyamus spp, the Lycium spp. Plant parts (stem. leaves. fruit and roots) were chromatographicly analyzed using TLC and GCMS techniques. Results were compared with results of same analysis for authentic standards of targeted alkaloids. It was conclude that Hyosyamus spp can be a good source for Atropine and Hyoscine for industrial use. The Lycium spp dos not contain addictive alkaloids and also do not contain significant concentration of toxic alkaloids such as Nicotine; it can be utilized for chemical, food and livestock feeding industries.

Keywords - Solanaceae family, Atropine, Hyoscine, Nicotine, Alkaloids extraction, GCMS analysis, TLC ,medical industry, Chemical industry,

I. INTRODUCTION

Alkaloids are a group of naturally occurring compounds that mostly contain basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties. Alkaloids are produced by a large variety of organisms including bacteria, fungi, plants, and animals (McNaught and Wilkinson 1997).

The Solanaceae, or nightshades, are an economically important family of flowering plants. Many members of the family contain potent alkaloids, and some are highly toxic, but many cultures eat nightshades, in some cases as staple foods (Olmstead 1999). The Solanaceae consists of about 98 genera and 2,700 species, with a great diversity of habitats, morphology and ecology. (Olmstead 2007).

Hyoscine and Atropine and Nicotine are substances of the most common alkaloids, they have highly toxic and narcotic effect. The main source of the first two types of alkaloids is the Datura spp from solanaceae family and the main source of Nicotine is Nicotiana spp.

This study focuses on detecting the presence of Hyoscine, Atropine and Nicotine in other plant species from the family solanceae than their known sources, namely the Hyosyamus spp, the Lycium spp, these species are found in wild environment in many countries in Africa and middle east .

Atropine: Atropine is a tropane alkaloid, which is an isomer of hyoscyamine, the source of atropine is some members of the solanaceae family (Burst John 2004), it found in different concentration in Datura stramonium, Hyoscyamus spp., Brugmansia suaveolens, etc. Atropine has two major actions: it is affecting the central nervous system (CNS). On the other hand this alkaloid depresses the smooth the muscle and the secretory glands which are innervate by the parasympathetic nerves (Asture and wandodkar 2008)

Historically atropine was used as cosmetic, it was used by the Greeks as an eye-drop to make the pupils expand and look seductive. Atropine eye drops are still used today, in small doses, to avoid eye problems which can lead to blindness (Thorpe 2015). Atropine used as a medication to treat certain types of nerve agent and also, some types of slow heart rate, and to decrease saliva production during surgery. It is also used as pesticide poisons (Richard et al. 2014).

Atropine molecular formula C17H23NO its molar mass is 289g/mol (Figure 1) , atropine is soluble in : 1g in 400 ml of water, 1g in 50ml of boiling water, 1g in 2ml of ethanol, 1g in 1ml of chloroform, 1g in 25ml of ether and 1g in 27ml of glycerol. It is not soluble in benzene and dilute acid (Anthony et al 2011).

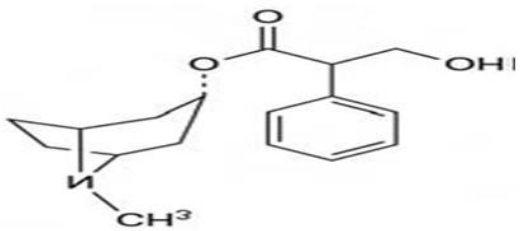


Figure 1 Atropine chemical structure

Hyoscine: Hyoscine is torpane alkaloid found in a variety of solanaceous plants, it is also known as scopalamin, hyoscine used as a medication to treat motion sickness and postoperative nausea and vomiting. It is also sometimes used before surgery to

decrease saliva (Gennaro et al. 1995). It found in Hyoscyamus niger, Brugmansia suaveolens, Datura stramonium. Hyoscine is a hallucinogenic substance if administrated in high doses can lead to coma or body death (Sweta and laksimi, 2015). These alkaloids exert two quite separate effects on mammals , an anticholinergic effect and central nervous system depressant effects. This combination of properties, their potent effects, in very small dosage, and their widespread distribution made them famous in the history of all cultures (Pearn , Thearle 1982).

Until the early 19th century, hyoscine was not separated as a pure substance, but occurred in extracts and tinctures containing a cocktail of plant-derived cholinergic blocking drugs (Pearn , Thearle 1982).

Hyoscine has the molecular formula is C17H21NO4 and molar mass of 303.4 g / mol (Figure 2.2), crystalline monohydrate, Soluble 1 in 9.5 of water , freely soluble in hot water, freely soluble in ethanol, chloroform, acetone and ether, sparingly soluble in benzene and Petroleum ether (Anthony et al 2011)

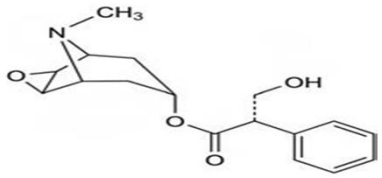


Figure 2 Hyoscine chemical structure

Nicotine: Nicotine is pyridine alkaloid found in the nightshade family of Solanaceae, and natural in all parts of the tobacco plant, with a high concentration in leaves, which constitutes 0.3 to 5% of plant dry weight. Its biosynthesis takes place in the roots and accumulating in the leaves. Nicotine is found in tobacco, tomatoes, potatoes, green papers, eggplants and other, it is also found in coca leaves. Plants, especially tobacco, used nicotine to defend themselves against insect (ujvary istvan 1999).

Nicotine has a lot of therapeutic uses. There's growing evidence that it may be useful in treating Parkinson's disease, Alzheimer's , Nicotine was widely used as an insecticide in the past(Rodgman, Perfetti 2009).

Nicotine has the molecular formula C10H14N12 and its molar mass is 162 g/mol (Figure 3) .It is colorless to pale yellow, very hygroscopic, oily liquid with an unpleasant pungent odor it gradually becomes brown on exposure to air or light. Nicotine is very soluble in ethanol, chloroform, petroleum ether, kerosene, oils and ether. (Anthony et al 2011).

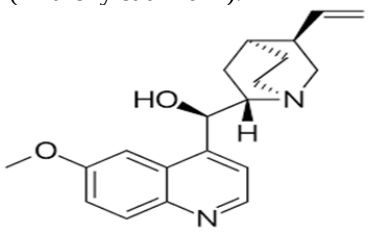


Figure 3 Nicotine chemical structure

Hyouscyamus albus L: Is a small genus flowering plants in the solanaceae family, it comprises twelve species, all of which are toxic. Hyoscyamus albus is known as (white henbanes) (Figure 4) and Hyoscyamus niger (black henbanes) (united states Department of Agriculture 2010). This genus can be easily identified in terms of phenotype and chemical composition (Liberman, Mitchell, 2008).

The Mediterranean basin is the main origin of this species of medicinal plants, the coastal stripes are overlooking southern Europe , and the Arab Maghreb, heavy wilderness species , however, proven and quality in deserts of Egypt and Libya in Africa , and west Punjab in Asia, Spain and Greece in Europe ,the main producing and exporting countries of henbane plant India , Afghanistan , Pakistan ,and Egypt .

Throughout history, it has been used alongside other plants as anesthetic and magic drinks for their psychologically influential characteristics such as. These include visual hallucinations, sense of conflict, fight, and quarrel. There are many type of henbane, which may vary in their contain of the type of alkaloids and their quantity, e g Egyptians henbane carries Hyoscine and Hyoscyanine, while black henbane and reticulates henbane, they produce both the former alkaloids next to Atropine. The results of the chemical analysis show that Egyptian henbane contains the highest amount of alkaloids except Atropine compared to the black henbane and reticulates henbane (Raetsch 2005). Hyoscyamus albus extracts were used in traditional medicine as an antiasthmatic and antispasmodic. It was also used as hallucinogenic and Sedative alone or mixed with Cannabis and Datura (Ali Esmail 2018)



Figure 4 Hyusyamus albus

Lycium albus: Lycium is a species of the family Solanaceae, it is a flowering plant distributed in most continents in temperate and subtropical regions. The largest number of species are found in South America, North America and South Africa. Some species are found in Europe and Asia (Fukuda 2001).

There are about 70 to 80 species. The most important is Lycium albus(Figure 5) , Lycium barbarum and Lycium chinens which is the fruit of an important traditional food crop in China and has recently become a popular health food worldwide (Levin, and Mille 2005).

Lysium has been known for European since ancient times. Species from the Far East were traded to Europe by the Romans, for example via Ariaka and Barbarikon Harbor near Karachi, This type of plants was recommended for treatment of eye inflammation and skin infections, (Lenaeus 1753) describes three species: Lycium: L. afrum, L. barbarum and L. europaeum. Historically Lycium has long been known, especially L. barbarum which has been used in traditional Chinese medicine to treat certain cases such as male's infertility. The leaves and roots of other types of lycium, have been used after mixing with water to treat skin rash and promote hair growth (Luo 2006).



Figure 5 Lycium deserti phil L

II. MATERIAL AND METHOD

Materials:

- Hyoscyamus albus samples collected from Dungula, Northern state. Sudan.
- Lycium deserti pil L samples collected from Arkaweet, Red see state. Sudan.
- Standard sample of atropine purchased from sigma - Aldrich.
- Standard sample of Nicotine was purchased from sigma-Aldrich.
- Standard sample of hyoscine was purchased from sigma-Aldrich.

Samples preparation: Plant sample were divided into plant part (roots, stems, leaves and fruits) then dried at room temperature and grained into powder.

Preparation of standards samples: Atropine standard was prepared by adding 1 ml of methanol (HPLC grade) to 1 g of atropine. Hyoscine standard and nicotine standard were prepared by adding 1 ml of methanol to 1 ml from hyoscine and 1 ml of nicotine.

Methods of Extraction of Atropine Hyoscine and Nicotine: 1000 mg of herbal powder of each sample of plant part of targeted plant were weighed; 1ml of chloroform was added to the powder of each sample and left for 24 hour at room temperature. The suspensions were filtered (Anthony et al 2005).

Thin layer analysis (TLC): Samples of part of each plant extract were loaded in TLC plate. A control sample of Atropine, Hyoscine, Nicotine were loaded in the plate beside the plant samples, each plate used for detection of one type of target alkaloids. The plates were but in TC system (mobile phase chloroform 90% methanol %10 in saturated condition) the mobile phase run for 10 cm. The RF was observed under ultraviolet radiation at a wavelength of 254nm (Anthonye et al 2005).

Gas chromatography analysis with mass spectrometer GC MS: Positive results samples were evaporated under Nitrogen, the dried residues dissolved in solution of (methanol: acetic acid 9:1). The samples were injected in GCMS chromatograph (Shimatzu 4080). The Injection volume was 1 ul, the start temperature of the column was 80c hold for 1 minute, then increase to 200 C at a rate of 15dgree per minute and hold for 1 minute. Then increased to 260c at a rate of 10 degree per minute and hold for 1 minute, and then increased to290c at a rate of 10 degree for 2 minute.

III. RESULTS AND DISCUSSION

Results of TLC analysis:

At 10 cm run for the mobile Rates of flow RF for the standard samples of atropine, hyoscine and nicotine were 2, 5.3 and 3.6 respectively.

All plant parts of Hyoscyamus albus except the root have shown positive result for atropine hyoscine, while the only positive result for nicotine was obtained from leaves samples. This is in agreement with The US Botanical and Plant Chemistry Database (2004)

A positive result for Nicotine was observed in the leaves sample of Lycium deserti phil, that is in good agreement with (Matthews 1994).

Result of GCMS analysis:

GCMS analysis of alkaloids standards:

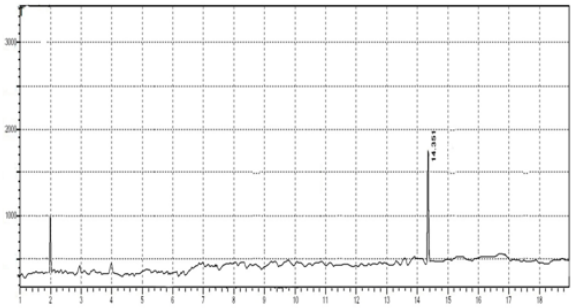


Figure 6 GC chromatogram of Atropine standard

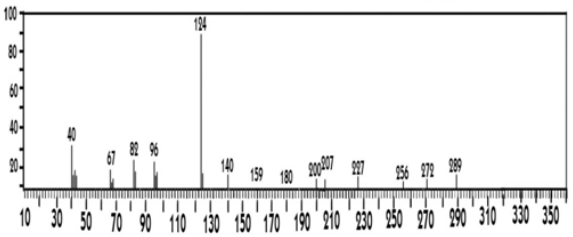


Figure 7 Mass Spectra of Atropine

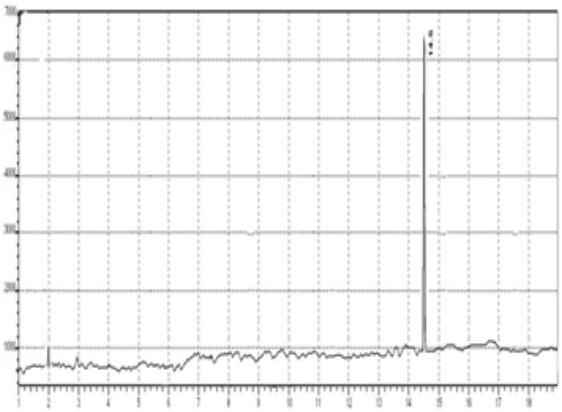


Figure 8 GC chromatogram of Hyoscine standard

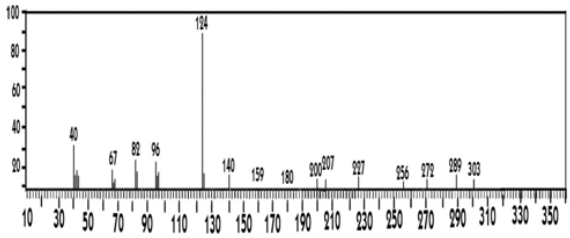


Figure 9 Mass Spectrum of Hyoscine

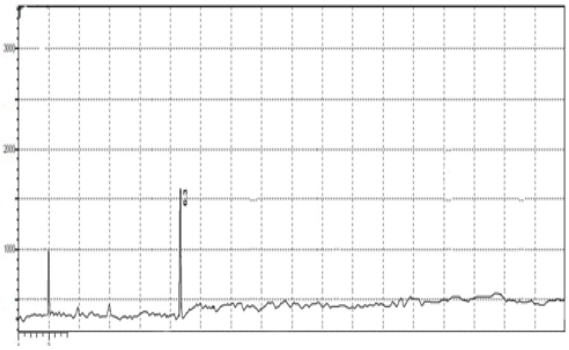


Figure 10 GC chromatogram of Nicotine standard

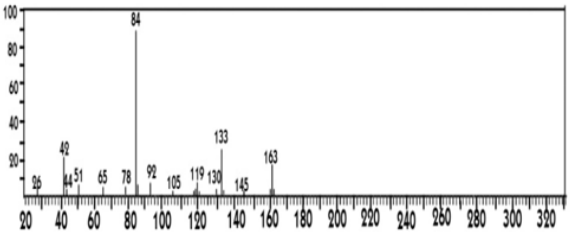


Figure 11 Mss Spectra of Nicotine standard

Number	Retentions time (minutes)	Standard sample	Standard peak height
1	14.3	Atropine	1711480
2	14.5	Hyoscine	6793721

Table 1 Retentions time (RT) and peaks heights of standard samples

Results of Hyoscyamus alpus plant parts:

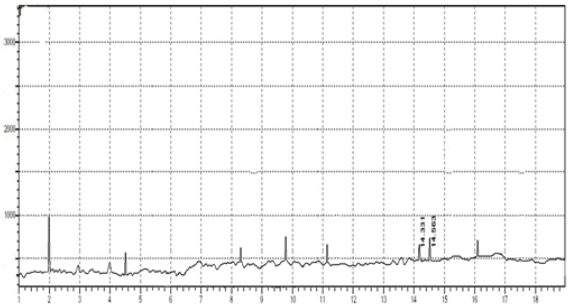


Figure 12 chromatogram of H. albus stem sample analysis

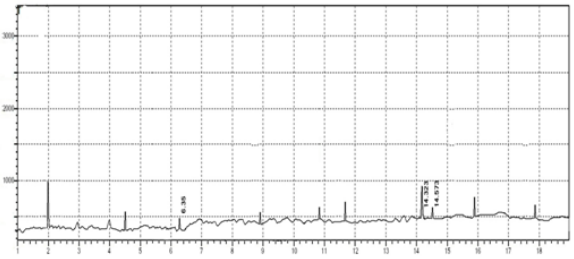


Figure 13 chromatogram of H. albus leaves sample analysis

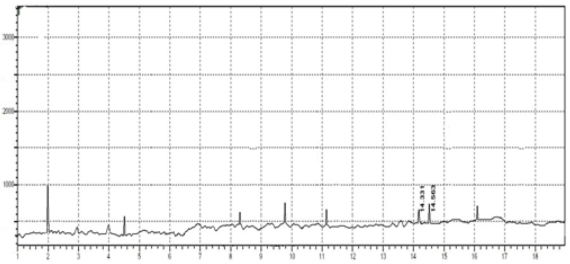


Figure 14 chromatogram of H. albus fruits sample analysis

Table 2 peak heights of H. albus plant part samples

Plant part	Peak height		
	Atropine	Hyoscine	Nicotine
Stem	52457	50374	-
Leaves	74257	37743	37437
Fruits	375324	39245	-
Roots	-	-	-

The chromatogram of the stem sample of Hyoscyamus albus (Figure 12) showed a peak at Retention Time of 14.3 minutes, the mass spectra of this peak was in the pattern of Atropine, the same sample chromatogram showed a peak at Retention time of 14.5 minutes. The mass spectrum of this peak agrees with the mass spectra of the Hyoscine standard. This was confirming the presence of the Hyoscine in the sample. The leaves chromatogram sample of Hyoscamus albus (Figure 13) shows a peak at 14.32 minutes the mass spectrum of this peak sample was in agreement with the mass spectrum of the standard sample of atropine, which confirms the presence of Atropine in the sample. The same sample chromatogram showed a peak at 14.57 minutes the mass spectrum of this is confirms the presence of Hyoscine. In the same chromatogram at

Retention time 6.32 minute a peak was observed the mass spectrum of this peak is similar to the mass spectrum of the Nicotine standard which confirms the presence of Nicotine. The fruits sample of Hyoscyamus albus was analyzed; the GC chromatogram(Figure 14) shows two peaks at Retention times 14.37 and14.58 minutes the mass spectra of these peaks was in agreement with the spectra of Atropine and hyoscine indicating the presence of these alkaloids in the sample.

Results of Licium deserti phil plant parts:

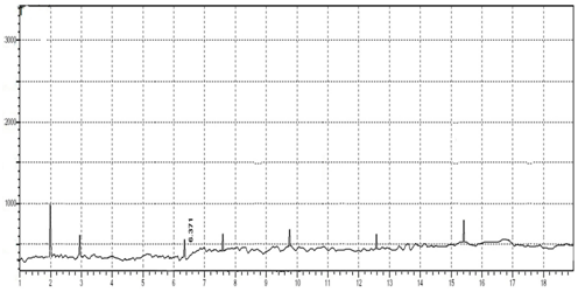


Figure 15 Chromatogram L. deserti phil leaves analysis

Plant part	Peak height		
	Atropine	Hyoscine	Nicotine
Stem	-	-	-
Leaves	-	-	47654
Fruits	-	-	-
Roots	-	-	-

Table 3 peak heights of L. deserti phill plant part samples

Figure 15 is showing the chromatogram of analysis for leaves sample of lycium deserti phil a peak was observed at Retention time of 6.37 minutes . The mass spectrum of this was similar to the Nicotine standard, which confirms the presence of Nicotine in the sample.

Plant part	Concentration mg/g		
	Atropine	Hyoscine	Nicotine
Stem	0.03034	0.07414	-
Leaves	0.04295	0.07414	0.022411
Fruits	0.2171	0.02375	-
Roots	-	-	-

Table 4 Concentrations of targeted alkaloids in H. albus plant parts

Plant part	Concentration mg/g		
	Atropine	Hyoscine	Nicotine
Stem	-	-	-
Leaves	-	-	0.02855
Fruits	-	-	-
Roots	-	-	-

Table 5 Concentrations of targeted alkaloids in L. deserti phill plant parts

Hyosyamus albus (Table 4) concentrations showed relative concentrations to Dtura spp. Atropine was in concentrations in rang of 0.03, 0.04 and 0.02 mg/g for stem, leaves and fruit respectively Eeva et al 1998 reported semilar results . Hyoscine concentrations were in the range of 0.07 mg/g in stem and leaves, the concentration in fruits was in the range of 0.02mg/g .which was in agreement with what was reported by (Alaghemand 2013). Leaves was contained 0.02 mg/g compatible with (Tadeusz 2015).

Table 5 is showing the concentrations collected from Lyceum deserti phil. Nicotine was accumulated in leaves in range of 0.01 mg/g as a characteristic phenomenon for family Solanaseae family. (Tadeusz 2015).

IV. CONCLUSION

The specimens of Hyosyamus albus which were analyzed can be utilized as a good raw material for medical and chemical industries.

Lycium deserti phill specimens were clear of toxic addictive alkaloids; they contain Nicotine in non significant concentrations for medical industry utilization, while it can be utilized for chemical, food and livestock feeding industries.

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