



Research Article

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Quantitative analysis of Hyoscine in different extracts obtained from the seeds of *Datura innoxia* by RP- HPLC

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ABSTRACT

India has a great wealth of various naturally occurring herbal drugs which have great potential pharmacological activities. *Datura innoxia* is one among such ornamental herb belongs to the family Solanaceae, which bears a beautiful white, purple or yellow color, single or double blossoms flower. From ancient times continuing to the present, especially considering the *Datura* spp., that to be seeds, it was used in shamanistic rituals as a path to enlightenment. Solanaceae family which is of great economic importance, is one of the largest flowering plant families with about 2,300 species. Besides this, the family is also extremely important as a source of drugs in medicine such as in the treatment of skin eruptions, colds, nervous disorders, narcotic for surgical procedures, anti-spasmodic, anti-asthmatic, narcotic, anti-microbial agent and neuro-sedative, but many are poisonous when used in excess. The phytochemical investigation concluded that the leaves are rich in atropine alkaloids such as scopolamine, hyoscyamine, hyoscine, norscopolamine, meteloidine, flavonoids, cardiac glycosides, essential oils, saponins and phenols. Today, people frequently experiment with it for the hallucinogenic effect, but the results are so unpleasant (dark visions, disorientation, amnesia, blurred vision, dry mouth, and incontinence) that they seldom recommend the experience. So in this context objective of the current review was to investigate the hyoscine content in different extract prepared with chloroform, ethyl acetate and methanol. The quantitative estimation of hyoscine in different extract was measure by RP-HPLC using PDA detector. The experimental report shows documentary evidence that, the concentration of hyoscine is maximum in chloroform and lowers in methanolic extract.

Keywords: *Datura innoxia*, hyoscine, RP-HPLC.

INTRODUCTION

Medicinal plants are traditionally used to prevent and treat various diseases [1]. Despite the development of advanced methods of treatment, still mortality rate is increasing every year [2, 3]. Medicinal plants are one of the most important sources for medicinally active compounds [4, 5]. One of such potent medicinal herb is "*Datura innoxia*". *Datura* belongs to family Solanaceae describe in Ayurveda for the various types of treatments to cure disease conditions in classical texts. The compounds from the Solanaceae that play such a prominent role as therapeutic drugs are all types of tropane alkaloids [6]. A large number of tropane alkaloids are known for pharmacologically importance, in which the nitrogenous base is esterified with tropic acid (or its derivative) are apparently unique in this family [7]. However, only three compounds have attained worldwide eminence as chemotherapeutic agents viz: L-hyoscyamine, L-hyoscine (Scopolamine) and atropine [8]. Other related but more recently discovered compounds, which are assuming growing importance, include anisodamine and anisodine (dataramine) [9]. The Solanaceous tropane alkaloids especially hyoscine are considered, after the indole alkaloids, to be the most important hallucinogenic compounds [10]. Devil's trumpet also contains a host of phytoactive chemicals including atropine, hyoscyamine, scopolamine, norscopolamine, meteloidine, hydroxy-6-hyoscyamine, tiglic esters of dihydroxytropine, and a number of withanolides [11-14]. Besides above this herb also contains other bio-potent medicinal active compounds such as oleic acid, palmitic acid, scopolamine, acetic acid, acetone, 27 acotonic acid, aesculetin, caffeic acid, chlorogenic acid, scopoline, pyridine and succinic acid [15-17]. Traditionally this herb plays a significant role in the health care system due to its hallucinogenic property [18]. The fruit juice is applied to the scalp for the treatment of falling hair and dandruff [19]. It is applied to smooth the painful wounds and sores [20]. Hyoscine is one of the important tropane alkaloids possess the cholinergic-blocking activity and it is used to calm schizoid patients and drug of choice in motion sickness [21]. The leaves of this herb contain hyoscine, hyoscyamine and atropine, which are used as an immensely powerful mind-altering drug [22]. The seeds of the plant are medicinally most active, which possess the therapeutic activities such as analgesic, anthelmintic and anti-inflammatory and as such, they are used in the treatment of stomach and intestinal pain that results from worm infestation, toothache, and fever from inflammation [23]. Seeds of this plant were used to sedate hysterical and psychotic patients, also to treat insomnia [24]. It is also used to smooth muscles relaxant of the bronchial tube and asthmatic

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bronchial spasm [25].

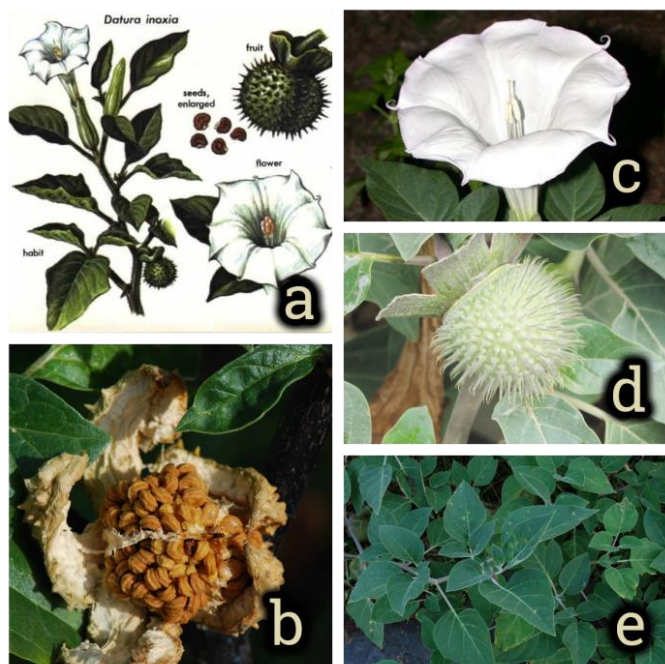


Figure 1: (a) Schematic diagram of plant with different parts, (b) seed, (c) Flower, (d) Fruit, (e) Leaves

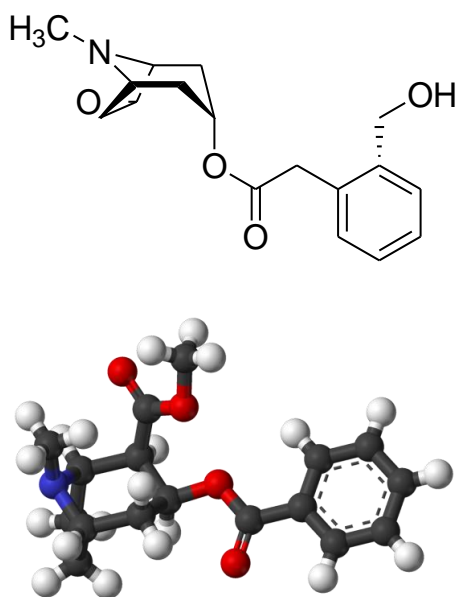


Figure 2: Chemical structure of hyoscyine

MATERIALS AND METHODS

For present study, the dried seeds of *Datura innoxia* were collected from the local market of Jammu, were authenticated by Dr. S. N. Sharma, Taxonomist at IIIM, Jammu, India.

Preparation of alkaloidal rich extracts

200gms of dried seeds *Datura innoxia* were crushed to coarse powder, and packed in percolator. The plant material was defatted with petroleum ether (40-60°C) for 48hours for removal of non-polar moieties like fats and oils. The residue left was packed in a soxhlet extractor and continued extraction with methanol. The extraction was continued for 72hour and concentrated with the help of thin film evaporator. The methanolic extract was triturated with 5% v/v of 0.5N HCl. The aqueous fraction was basified with ammonia solution up to pH

9. Then the solution was portioned with chloroform. The process was repeated for 5-6 time till the chloroform layer gets discolored. The remaining aqueous layer was further fractioned with ethyl acetate. Similar protocol was followed as in chloroform. At last the different fractions obtained were concentrated with the help of thin film evaporator. The different solid extracts were weighed and store in a desiccators for future use. The whole protocol was shown in fig. 3. The different extract was subjected for preliminary phytochemical analysis for the presence of different secondary metabolites [26, 27].

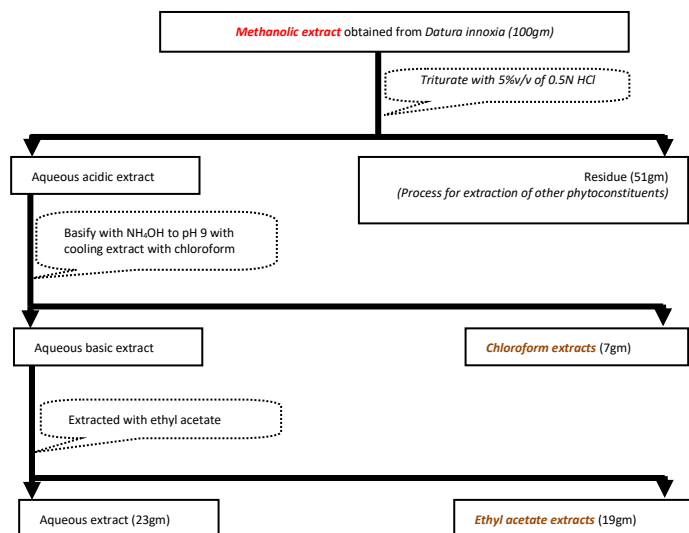


Figure 3: Schematic diagram of preparation of alkaloidal rich extract

Isolation of phytoconstituents

Chloroform extract of was considered for isolation of phytoconstituents. The extracts was dissolved in the minimum quantity of chloroform and then adsorbed on silica gel, to get free flowing material. The slurry was loaded in a column of 110cm long and 5cm diameter (ID). The column was initially eluted with petroleum ether and gradually eluted with increasing the polarity with ethyl acetate. A 100ml fraction was collected and monitored with TLC. A total of 200 fractions were collected. Fraction no. 72-119 eluted in 30% ethyl acetate in petroleum ether shows a single spot in TLC with rf value 0.46 with mobile phase (chloroform: methanol:: 95:5). The pure compound was characterized by considering the various physical and analytical techniques. Reviewing the literature survey and comparing data, it was concluded that the isolated compound was hyoscyine [28, 29].

Quantitative analysis hyoscyine in different extract by RP-HPLC

Sample preparation: 10mg of hyoscyine was weighed accurately and transferred to a 10 ml volumetric flask and dilution was made with methanol (HPLC grade). One more dilution was made to get a working stock solution of 100 ppm. The working standard solution was prepared from the stock solution by transferring 1, 2, 3, 4 and 5 ml solution to 5 different 10ml volumetric flask and the final volume was made with methanol to get a concentration of 10, 20, 30, 40 and 50 ppm.

HPLC System

A waters HPLC system consisting of two pumps waters 515 HPLC with waters pump control module, an automatic sampling unit waters 717 plus auto sampler, a column oven a photodiode array detector waters 299 6 and temperature control module II. Waters Empower software was used for data analysis and data processing. The samples were analyzed at 30°C on a Merck RP-18 column (5µm, 250 x 4.0 mm ID) by UV detection at 340 nm. The mobile phase consists of acetonitrile (ACN): 1.5%v/v acetic acid (AcOH) in distilled water was delivered at a flow rate of 1mL/minute. The retention time of the is found to be (Rt)

16.86 min. A calibration curve was plotted by considering the concentration of 10 to 50 ppm with a regression of coefficient ($r^2 = 0.999$). A 20 ppm solution of the different extracts were prepared and injected to HPLC system and area under the curve was recorded.

RESULTS AND DISCUSSION

Different extracts were prepared from seeds of *Datura innoxia* with different extracting solvent like methanol, chloroform and ethyl acetate. The chloroform extract was subjected to column chromatography for isolation of active chemical constituents. One of

the isolated compounds was designated as hyoscyne. The compound was characterized by physical and instrumental techniques like solubility study, melting point, Tlc, UV visible Spectrometry and HPLC. The preliminary phytochemical investigation of different extracts was shown in Table no 2. The different extracts were standardized by RP-HPLC. The ethyl acetate extract contains 0.80% w/w of hyoscyne followed by methanolic extract which contains 0.22%w/w and least amount was found in chloroform extract. The chromatogram of different extracts outlined the concentration of hyoscyne content (Fig. 2).

Table 1: Hyoscyne content in different extracts

Sl. No.	Extract Name	Color	Texture	Retention Time	Quantity (%w/w)
1.	Chloroform extract	Golden	Powder	16.85	0.80
2.	Methanolic extract	Brown	Powder	17.14	0.05
3.	Ethyl acetate extract	Brown	Semisolid	17.01	0.22

Table 2: Preliminary phytochemical analysis of different extracts

S. No.	Tests	Chloroform extract	Ethyl acetate extract	Methanol extract
1.	Alkaloids	+	+	+
2.	Carbohydrates	+	+	+
3.	Gums/Mucilage	-	-	-
4.	Tannins	-	+	+
5.	Flavonoids	-	+	-
6.	Saponins	+	+	+
7.	Sterols	+	+	+

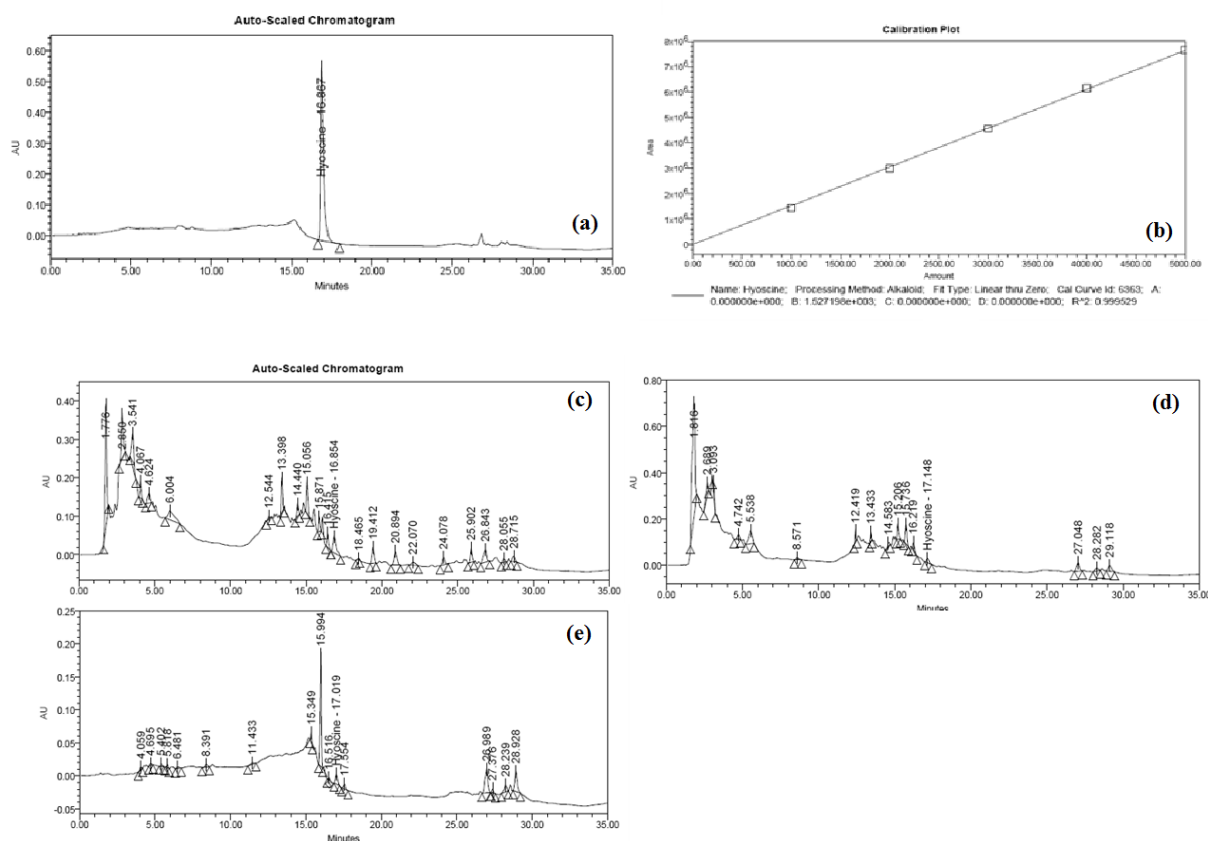


Figure 4: (a) Chromatogram of isolated hyoscyne, $R_t=16.86$ min, (b) Calibration curve of hyoscyne by considering the different concentration of 10, 20, 30, 40 and 50 ppm and absorbance are recorded at 340 nm (c) Chromatogram of chloroform extract, (d) Chromatogram of methanolic extract, (e) Chromatogram of ethyl acetate extract

CONCLUSION

Datura innoxia has been known from ancient times to contain bioactive components that act as stimulants, depressants or hallucinogenic agents. This herb contains alkaloids that are useful in medicine and are not generally consumed as foods. In this research, a simplified and fast chromatographic technique like HPLC was outlined for the analysis of hyoscyine content in seed extracts of *Datura innoxia*. The result of the research gives a significant detail on hyoscyine content in the different extracts, extracted with different solvent like chloroform, ethyl acetate and methanol. It was found that the percentage of hyoscyine content in maximum in chloroform extract followed by ethyl acetate extract and least in methanolic extract. No doubt, hyoscyine is a masterstroke for the treatment of motionsickness, postoperative nausea and vomiting but still it has some side effects like sleepiness, blurred vision, dilated pupils, and dry mouth. It is not recommended in people with glaucoma or bowel obstruction. In this context this article also emphasize to formulate a standardized bioactive extract rather giving importance pure compound, which may leads diminish its side effect to some extent.

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Conflict of Interest: None Declared.

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