ISSN: 2278-4632
Vol-10 Issue-6 No. 13 June 2020

Extraction and Identification of atropine in *Datura stramonium* seeds using Thin layer chromatography

Hiramanee Patidar[*], Dr. Kavita Sharma[**]
[*] Research Scholar, SVIFS, Shri Vaishnav Vidyapeeth Vishwavidyalaya, Indore M.P.
[**] Professor, (Chemistry), SVIFS, Shri Vaishnav Vidyapeeth Vishwavidyalaya, Indore M.P.
Email Id- manimani.h10@gmail.com, kavitasharma5872@gmail.com

Abstract – Datura is an annual herbaceous plant of the solanaceae family. It is widely distributed and easily accessible. *Datura stramonium* contains toxic alkaloids. The main toxic alkaloids are atropine, scopolamine and hyoscyamine. It is used in Ayurvedic medicine to cure so many diseases such as rheumatism and gout, sciatica, bruises, fever, asthma etc. It has been also used in religious rituals. The seeds of *D. stramonium* are used to abuse to achieve a hallucinogenic effect. It is very toxic, sometimes accidental poisoning of humans and animals also has been reported. This research paper was an attempt to extract and identify atropine from seeds of *D. stramonium*. The alkaloids extracted by the maceration method. Color tests and thin layer chromatography techniques were used for the identification of atropine. The results showed the presence of atropine in *Datura stramonium* seeds.

Keywords: *Datura stramonium*, Maceration, Alkaloid, Extraction.

Introduction –

Plants are the most important part our ecosystem. Both humans and animals depend on plants directly and indirectly. Plants are source of food, medicinal treatment, decoration, and many other purposes. It is an annual herbaceous plant belongs to the genus Datura and solanaceae family comprises all the nightshade and agricultural plants including potato, tomato, coffee, and pepper. *Datura stramonium* is a very common species grown in the tropical and subtropical region of the world mainly in Iran, Mexico, Canada, India, America, and Southeast Asia, North Africa, etc. various species of Datura's are *Datura stramonium, Datura inoxia, Datura forox, Datura metel, Datura fastuosa,* etc. The common name of *Datura stramonium* is Jimsonweed, devil's weed, angel's trumpets, devil's trumpets, moonflowers, apple of Peru, thorn-apple, hell's bells. The major importance of datura is in Ayurvedic medicine for the treatment of fever, ulcer, wound, sciatica, rheumatism and gout, inflammation, bruises and swellings, toothache, asthma, and bronchitis. In Hindu religion *Datura stramonium* traditionally and myth-logically is believed to be an affiliation with the God Shiva. The whole plant of *Datura stramonium* is poisonous in nature, due to the presence of alkaloids such as atropine, hyoscyamine and hyosine. Because of

ISSN: 2278-4632
Vol-10 Issue-6 No. 13 June 2020

easy availability of this plants misuse has been also widely embraced in cases of homicidal and suicidal poisoning [7].

Materials and Method –

Sample Collection and Preparation –

A fresh plant sample of *Datura stramonium* was collected from Patidar Colony Jamner block Shujalpur District Shajapur Madhya Pradesh. The plant sample was collected, cleaned and manually separated. The capsules were dried at room temperature for 10 to 12 days. After dried the spiny capsules seeds turned into brown and black in color. The sample seeds grinded by pestle mortar to get fine powder of the seeds and then allow it to passing through 60 ASTM sieve.

Preliminary Examination of Alkaloids – The extract was used for different color tests (Wagner's test, Dragondorf's test, Mayer's test) for the identification of alkaloids.

Preparation of the plate for Thin layer chromatography Examination -

The glass plate was carefully rinse with distilled water and air-dried then wiped with acetone to remove oil and grease. The slurry prepared using silica gel G was immediately spread on glass plate. The plate was air-dried for 10 minutes on alignment and activated by heating in an oven at 110°C for 30 minutes. The solvent system depends on the properties of the desirable components to be separated. The concentrated extracted sample was loaded on approx 1cm distance of chromatographic plate through a capillary tube. [15]

Solvent System – The solvent system used for identification of the alkaloids

Acetone: Distilled water: concentration Ammonia (90: 07: 03)

Methanol: Distilled Water (70:30)

Methanol: Ammonia (98:2)

Method –

In *Datura stramonium* seeds many phytoconstituents and different functional groups present such as Steroids, flavonoids, phenols, glycosides, alkaloids, oils, fats, terpenes, wax and many others. In this work extraction and identification of atropine alkaloids was done from seeds of *D. stramonium*. The other contains like oils, fats, terpenes, wax etc, where removed from the seeds by using petroleum ether. The 5gm of fine powder of seeds and 30 ml of Petroleum ether was taken in conical flask and shaken to remove other contains. This sample was hold approx five

ISSN: 2278-4632
Vol-10 Issue-6 No. 13 June 2020

hours to remove other undesired phytoconstituents. The sample was filtered through whatman filter paper. After treatment with petroleum ether, in sample add 60 ml ethanol and 100 ml n-hexane was added separately in 250ml conical flask. The flask was covered with cotton, plastic or glass stock and aluminum foil. Extraction was performed for seven days. The agitation was randomly done at an interval of 12 hours. The soluble extract was collected by filtration through a 1mm diameter of filter paper. Filtered extract was concentrated and stored in refrigerator for further analysis. [1][11]

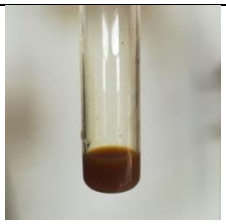
Thin layer chromatography Examination –

Three solvent systems (Acetone: Distilled water: concentration Ammonia (90: 07: 03) , Methanol : Distilled Water (70:30), Methanol : Ammonia (98:2)) were used. The chamber was pre saturated with above mentioned solvent system to find out variation in Rf values in different mobile phase. The TLC plate was developed in chamber, when the solvent reached up to 2/3 of the plate. These were removed from the chamber and dried. Then the plate were observed under UV light (Wavelength 365nm). After that, the plates were sprayed with Dragondorf's reagent to develop the color spot.

Rf = distance of the solute / distance of the solvent

Color test of alkaloids –

Preliminary Examination of alkaloids

S. No.	Test Name	Color test	Extracted Sample (Seed)
1.	Wagner's test		Positive (Reddish-brown)
2.	Dragondorf's test		Positive (Reddish-brown)
3.	Mayer's test		Positive (Cream or Greenish)

[5, 13]

ISSN: 2278-4632
Vol-10 Issue-6 No. 13 June 2020

Rf value of different fractions of solvent extraction on crude extract from *D. stramonium* seeds

S. No	Solvent system	Seeds (Ethanol)	Seeds (n - hexane)
1.	Acetone : Distilled Water : Ammonia 90:7:3	0.87 0.65	0.85
2.	Methanol : Distilled Water 70:30	0.85 0.64	0.82
3.	Methanol : Ammonia 98:2	Tailing	Not clear

Results and Discussion –

Positive identifications were obtained from color tests on treating with Wagner's reagent reddish-brown color was observed. When treated with Mayer's reagent greenish or cream color and in addition to Dragondorf's reagents reddish brown color was observed. Rf values were calculated by observing results of chromatography from each plate with different solvent system. Ethanolic extraction of seeds was higher than n- hexane extract of seeds that shows Atropine presence in the ethanolic extract of the seeds. According to this research, the sample contain more polar biomolecules than non polar ones.

Conclusion –

The results present in the crude extract of *D. stramonium* seeds obtained from the maceration method using ethanol and n-hexane. Ethanol is the best solvent for the extraction of the atropine alkaloids compare to n-hexane and the maceration method for extraction purposes is cost-effective and efficient for the extraction of plant alkaloids from seeds. The suggested method is a simple and rapid method, any instrumental method is not required for analysis.

Reference –

1. F. Babiker, P. Jamal, M.E.S. Mirghani, A. H.Ansari, (2017), "Characterization, purification and identification of some Alkaloids in *Datura Stramonium",* International Food Research Journal 24 (Suppl); S540-S543.

ISSN: 2278-4632
Vol-10 Issue-6 No. 13 June 2020

2. Sramska Paula, Maciejka Artu, Topolewska Anna, Stepnowski Piotr, Halinski Łukasz P. (2016),"Isolation of atropine and scopolamine from plant material using liquid-liquid extraction and EXtrelut® columns", Journal of Chromatography B, 80-308.
3. Altameme Huda Jasim, Hameed Imad Hadi and Kareem Muhanned Abdulhasan, (2015), "Analysis of alkaloid phytochemical compounds in the ethanolic extract of Datura stramonium and evaluation of antimicrobial activity", African Journal of Biotechnology, Vol. 14(19), pp. 1668-1674.
4. Welegergs Gebregziabher Giday, Hulif Kalyou, Mulaw Solomon, Gebretsadik Haftu, Tekluu Berihu, Temesgen Ayalew,(2015) "Isolation, Structural Elucidation and Bioactivity Studies of Tropane Derivatives of Alkaloids from Seeds Extract of Datura Stramonium" Science Journal of Chemistry, 78-83.
5. Altameme Huda Jasim, Hameed Imad Hadi and Kareem Muhanned Abdulhasan, (2015), "Analysis of alkaloid phytochemical compounds in the ethanolic extract of Datura stramonium and evaluation of antimicrobial activity", African Journal of Biotechnology, Vol. 14(19), pp. 1668-1674.
6. Prasad Gaire Bhakta, Subedi Lalita (2012), **"**A review on the pharmacological and toxicological aspects of Datura stramonium L.", Journal of Integrative Medicine.
7. Kakhia Ismail Tarek, "Alkaloids and Alkaloids Plants", Adana University –Industry Joint Research Center.1-479.
8. Soni Priyanka, Siddiqui Ahmad Anees, Dwivedi Jaya, Soni Vishal, (2012)," Pharmacological properties of *Datura stramonium* L. as a potential medicinal tree: An overview", Asian Pacific Journal of Tropical Biomedicine (APJTB), Vol 2,1002-1008.
9. Hosseini Naser, Ebrahimi Samad Nejad, Salehi Peyman , Asghari Behvar and Ahmadi Mahmood (2011), "Simultaneous determination of atropine and scopolamine in different parts of Hyoscyamusarachnoideus Pojark plants by high-performance liquid chromatography (HPLC)", Journal of Medicinal Plants Research Vol. 5(15), pp. 3552-3557.
10. Bazaoui Ahmed El, Bellimam Ahmed My, Soulaymani Abdelmajid.(2010), "Nine new tropane alkaloids from Datura stramonium L. identified by GC/MS", Fitoterapia,193-197.
11. Djilani A. Legseri B. ,Soulimani R., Dicko A., and Younos C. , (2006), "New extraction technique for alkaloids", Journal of The Brazilian chemical Society, 17:518-520.

ISSN: 2278-4632
Vol-10 Issue-6 No. 13 June 2020

12. Iran bakhsh Ali reza, Oshaghi Mohammad Ali, and Majd Ahamad (2006) "Distribution of Atropine and Scopolamine in Different Organs and Stages of Development in Datura Stramonium L. (Solanaceae).Structure and Ultra structure of Biosynthesizing Cells". Act a Biological Cracoviensia Series Botanica 48/1.
13. Steen kamp P.A., Hardinga N.M., Heerden F.R. van Wyk B.-E. van, (2004), "Fatal Datura poisoning: identification of atropine and scopolamine by high performance liquid chromatography/photodio dearray/mass spectrometry", Forensic Science International 145 31–39.
14. Evans WC(2002), "Trease and Evans pharmacognosy", 15th edition, W.B. Sauders company Ltd, London, pp 137-139,230-240.
15. Danielson Neil D, Gallagher Patricia A, Bao James J, (2000), "Chemical Reagents and Derivatization Procedures in Drug", Encyclopedia of Analytical Chemistry R.A. Meyers (Ed.) pp. 7042–7076.
16. Takahashi Misako, Nagashima Machiko, Shigeoka Sutemi, Nishijima Motohiro, Kamata Kunihiro, (1997) "Determination of atropine in pharmaceutical preparations by liquid chromatography with fluorescence detection", Journal of Chromatography A, 775 137–141.
17. Gouria A.N. (1980),"The investigation of the alkaloids of the Datura stramonium crown in Kenya", Department of Pharmacy, University of Nirocty Kenya.

