CHAPTER I - INTRODUCTION

Forensic toxicology is a multidisciplinary field that involves the detection and interpretation of the presence of drugs and other potentially toxic compounds in bodily tissues and fluids. This acts as defensible evidence in the court of law. Forensic toxicology continues to be a dynamic field with evolving technology applications. Forensic toxicologists take care of the science of poison detection in human organs and body fluids.

Datura is a genus of nine species of vespertive flowering plants that belongs to the family of Solanaceae. They are commonly known as Datura but also known as Devil's trumpet, and also Jimsonweed, Devil's weed, Thorn apple and moonflower. There are nine species of Datura worldwide of which *Datura metel* is distributed widely in Asia and in Africa. As all Datura plants contain tropane alkaloids such as Atropine, Scopolamine, and Hyoscamine which are primarily present in the seeds and flowers. The word Datura comes from the early Sanskrit word dhattūra or Sanskrit Dustural or dahatura meaning white thorn apple originally published by Linnaeus in 1753.[18] There are several species of Datura that belong to the Solanaceae family and those species are as follows:-

- *Datura metel* commonly known as devils trumpet
- *Datura stramonium* also called as jimsonweed or thorn apple
- *Datura innoxia* also known as toloache, moonflower
- *Datura wrightii* also called as sacred datura
- *Datura ferox* commonly known as long spined thorn apple
- *Datura discolor* called as desert thorn apple
- *Datura ceratocaula* called as torna loco
- *Datura leichhardtii* also called as Leichhardt's
- *Datura quercifolia* known as oak leaf thorn apple [13]

In ancient Indian literature Datura is referred to as Shivashehara because the flowers are believed to be associated with Lord Shiva also used in rituals and prayers to Shiva. In Hindu mythology, Lord Shiva is the God of Destruction. He lives the life

of hermit in the wilderness of Mount Kailas and is clad in animal skin. He loves all wild things and thus pretty flowers are never offered to him. Datura is believed to have appeared from the chest of Lord Shiva when he drank the poison that was churned out of the cosmic ocean by the Devas and Asuras, for the welfare of the world. Therefore, Datura is offered to Lord Shiva to get rid of poison of envy, ego, rivalry, foul language and wicked nature so that one is fully cleansed of all his sins and become pure. The plant is attributed as poisonous and aphrodisiac. One of most powerful deliriant and hallucinogen to people living in the rural area and urban area of many places. These plants though serve medicinal values the toxicity of the plant is more compared to the medicinal value of the plant. [8]

In Ayurveda in some quantities Datura has medicinal properties such as analgesic, anthelmintic and anti-inflammatory and as such they have been used in the treatment of stomach and intestinal pain that is resultant of worms' infestation, toothache and fever from inflammation. The juice of its fruit is applied to the scalp, to treat the dandruff and hair fall. On external application, it improves skin quality and cures various skin disorders also this helps in quick wound healing. *Datura metel* is one of the 50 fundamental herbs used in traditional Chinese medicine, where it is called Yang Jin Hua. However, the ingestion of Datura metel in any form is dangerous and should be treated with extreme caution. According to Drug and Cosmetic Act 1940 and Rule 1995, *Datura metel* is banned in India for use in Ayurvedic medicine. Datura is used in preparing herbal plasters and also used as anodyne and anti-spasmodic.

All parts of Datura plants contain dangerous levels of highly poisonous tropane alkaloids and may be fatal if ingested by humans or other animals, including livestock and pets. Fatal dose of *Datura metel* is 50-70 seeds and the tolerance level makes the fatal dose to vary; fatal period is 24 hours. *Datura metel* may be toxic if ingested in a tiny quantity. Flushed skin, headaches, hallucinations and possibly convulsions or even a coma, anticholinergic toxicity and death in extreme cases are the symptoms. Oral administration of purified Datura seed is indicated in chronic respiratory disorders, asthma and dysuria. The plant contains belladonna alkaloids, predominantly atropine and scopolamine and the presence of at least 26 other related alkaloids will

vary even between specimens within the same species. The tropane alkaloids or belladonna alkaloids are spread from root to shoot of the plant. These alkaloids serve a major percentage of toxicity. The prominent symptoms are the blockage of peripheral muscarinic receptors that innervate the exocrine glands, smooth muscle and cardiac muscle. The primary toxic manifestations include mydriasis and cycloplegia which is due to the blockage of papillary sphincter muscle and iris muscle; dry mouth, secondary to parasympathetic blockage of salivary secretion; tachycardia, caused by competition at muscarinic receptors in postganglionic parasympathetic neurons and blockade of receptors is SA node: fever and erythema, because of the vasodilation and inhibition of sweating ; pupil dilations, dehydration changes in heart rate especially irregular or rapid beating.

The active toxic tropane alkaloid constituents present in the Datura species makes it to be abused as a hallucinogen. Datura is a hallucinogenic plant found in urban or rural areas across Asia and Africa that grows wild. This is thereby used as the tropane alkaloids presence gives hallucinogenic and euphoric effects. Reports say that teenagers ingest roots, seed or the entire plant as drug abuse due to its hallucinogenic effects. The higher concentrations of tropane alkaloids are present in seeds that are within the spiny fruit. The amount of seeds that produce hallucinogenic effects is less than the fatal dose and atropine serves most of the hallucinogenic effects when theses seeds are ingested this sis because atropine is an anticholinergic drug that blocks the action of neurotransmitter acetylcholine at synapses in the central and the peripheral nervous system. These agents inhibit parasympathetic nerve impulses by selectively blocking the binding of the neurotransmitter acetylcholine to its receptor in the nerve cell.

The chemical compositions of Datura are:-

1. Daturine (alkaloid)
2. Mucilage
3. Albumen
4. Tropane
5. Scopolamine

6. Hypocyamine
7. Hyoscamine
8. Hyoscine
9. Atropine
10. Norhyosciamine
11. Meteolodine
12. Malic acids
13. Scopolamine

The detection of the alkaloids of Datura metel from edibles serves a huge contribution to the cases that are very much common especially in rural areas. The Datura seeds are air dried and crushed into powders which is added into edibles like water, food and beverages, this act is often done for the commission crimes that are violent as well as non-violent the latter being more likely. These crimes maybe such as robbery, rape, murder, kidnapping, homicide and sometimes suicide and accident ingestion. The forensic significance of identification of Datura poisoned edibles is that it helps in the determining the cause of death or cause of poisoning by the analysis of the samples of the edibles, viscera samples and partially digested food. Another forensic significance of the identification of tropane alkaloids from the Datura poisoned edibles it that this identification can serve as corroborative evidence to the cause of death. Thereby coming down into a conclusion. The branch of knowledge concerned with medicinal drugs obtained from plants or other natural sources is known as **Pharmacognosy,** which is derived from two Greek words pharmakon meaning drugs and gnosis means knowledge deals with the natural drugs obtained from organisms such as most plants, microbes, and animals. Up to date, many important drugs including morphine, atropine, galanthamine, etc. have originated from natural sources which continue to be good model molecules in drug discovery.

There are major four steps of identification:-

i. Macroscopic identification
ii. Microscopic identification
iii. Organoleptic analysis

iv. Phytochemical analysis

Macroscopic identification is the general identification of the plant before analysis; this is the study on the morphological characteristics of the plants that are carried out by a naked eye or phytomorphology such as organoleptic features such as shape, size, colour, odour taste of leaves flowers and fruits were evaluated also the root, stem, leaves and phyllotaxy, inflorescence and flower with their aestivation and parts, fruits, seed and thereby describing the plant with the vegetative and floral characteristics and economic importance. This can be correctly identified by a botanist or with decent knowledge on botany.

Datura metel is identified from Aditya Degree College, Surampalem and Nilampathinjimugal, Kakkanadu. Botanical description on leaf, stem, fruit, and flower is done. The habit is large, erect and stout herb, with branched tap root system. The stem is hollow, green and herbaceous with strong odour. Leaf is simple, alternate, and petiolate, with reticulate venation. Flower is large, greenish white, purple in colour with 5 sepals and 5 petals showing valvate and twisted aestivation respectively. Fruit is spinescent capsule opening by four apical valves with persistent calyx inside which seeds are numerous that has a characteristic odour. This helps us to conclude that Datura belongs to the family of Solanaceae. The Datura sample collected was of two colours yellow-white and purple in colour. These two belong to the same species with two varieties.

The pictures of the plant was taken and after coming into a conclusion of the plant being the *Datura metel* plant were verified with a Ayurvedic medicine doctor who holds a PhD in Ayurveda for correct classification of the plant by collecting the plant from the sources. These plant samples were identified and by the doctor.

FIGURE 1.1 & 1.2 *Datura metel*

FIGURE 1.3 & 1.4 DRIED SEEDS AND FRUIT RESPECTIVELY

Microscopic identification is method in the microscopic level or cellular level of identification. In these types of identification the cross section of various parts of the plant is taken and visualised in various types of microscope starting simple microscope. In other words microscopic identification is the identification of unique characteristics that cannot be identified by using a microscope as they are invisible to naked eye unless aided by a microscope which is a simple tool or instrument that is

used to visualise objects in microscopic level. The Datura plant is microscopically identified by transportation vessels such as the xylem and phloem ; types of cells present throughout the plant, cross section of seed, leaves, stem, fruit, flowers, pollen grains, etc. Microscopic analysis increases the exclusivity and identities of the plants that has been identified by using macroscopic identification method and classify the plant species in rightly evaluating them. In this type of identification the choice of the suitable microscope in the identification is according to the complexity of the cellular level of the plant species as well as the maximum features that can be identified by the visualisation of the cells and distinguishing them from what is present in the slide prepared. For this slide preparation is very important and any error in the slide preparation may not reveal the features and this may lead us to start from the scratch. Another factor that depends upon the microscopic identification is that the instrument may be error free and calibrated well so as to obtain good results.

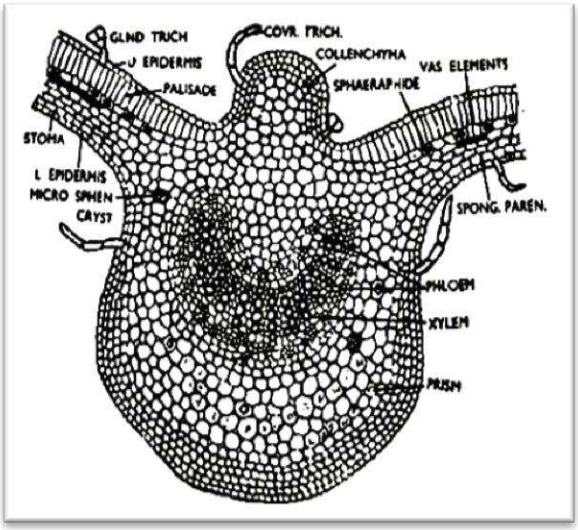


FIGURE 1.5 [16] & 1.6 [17] : T.S OF DATURA LEAF & DATURA

Organoleptic analysis or evaluation means the study of drugs using organs of senses. It refers to the methods of analysis like colour, odour, taste, size, shape and special features such as touch, texture etc. Obviously the initial sight of the plant or extract is so specific that it tends to identify itself. But this identification process is to be certain and absolute on to the specific drugs. These are random impressions. Datura

is bitter in taste and has a characteristic smell that is very strong and distinguishable from the other plants that has similar morphological characteristics. This type identification generalises and acts as a supporting identification.

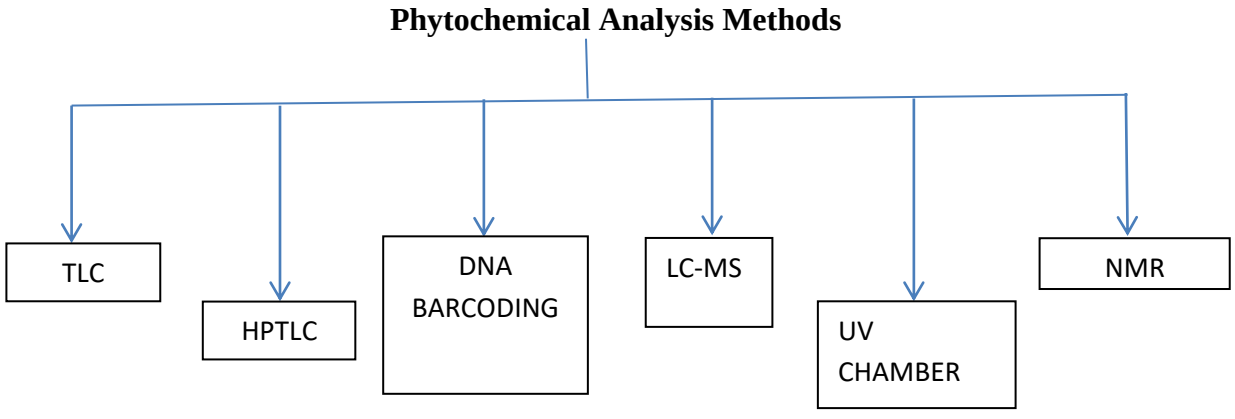
Macroscopic, microscopic and organoleptic identification is generally called as Pharmacognosy that is the study of medicinal drugs or generally drugs.

Constituents in general that are present in a plant are:

- Alkaloids
- Tannins
- Glucosides
- Amino acids
- Phytosterols
- Kaempherides

Phytochemical identification is process of treatment of live cells with chemicals or reagents or solvents for biochemical response with certain ingredients in the body. Phytochemical analysis is done to identify the constituents of a plant. The scopes of drug action are Pharmacokinetics, Pharmacodynamics, and Pharmacotherapeutics.

They are generally classified as:



Here will be using Thin Layer Chromatography, High Performance Thin Layer Chromatography and UV-VIS Spectrophotometer

THIN LAYER CHROMATOGRAPHY (TLC)

TLC is a simple, quick, and inexpensive procedure that gives the chemist a quick answer as to how many components are in a mixture. TLC is also used to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound (preferably both run on the same TLC plate). A TLC plate is a sheet of glass, metal, or plastic which is coated with a thin layer of a solid adsorbent (usually silica or alumina). A small amount of the mixture to be analysed is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid. This liquid, or the eluent, is the mobile phase, and it slowly rises up the TLC plate by capillary action. As the solvent moves past the spot that was applied, equilibrium is established for each component of the mixture between the molecules of that component which are adsorbed on the solid and the molecules which are in solution. In principle, the components will differ in solubility and in the strength of their adsorption to the adsorbent and some components will be carried farther up the plate than others. When the solvent has reached the top of the plate, the plate is removed from the developing chamber, dried, and the separated components of the mixture are visualized. If the compounds are coloured, visualization is straightforward. Usually the compounds are not coloured, so a UV lamp is used to visualize the plates. (The plate itself contains a fluorescent dye which glows everywhere *except* where an organic compound is on the plate.)

The retention factor, or R_f, is defined as the distance traveled by the compound divided by the distance traveled by the solvent

$$R_f = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent front}}$$

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

High Performance Thin Layer Chromatography (HPTLC) is the most powerful advanced form of Thin Layer Chromatography (TLC) and consists of chromatographic layers of utmost separation efficiency and the application of sophisticated instrumentation for all steps in the procedure include accurate sample application, standardized reproducible chromatogram development and software controlled evaluation. HPTLC is a concept that includes a widely standardized methodology based on scientific facts as well as the use of validated methods for qualitative and quantitative analysis. HPTLC meets all quality requirements for today's analytical labs, to increase the resolution and to allow more accurate quantitative measurements. Steps involved in HPTLC procedure

1. Sample Application
2. Chromatogram Development
3. Derivatization
4. Evaluation: Detection
5. Evaluation: Documentation

UV- VIS SPECTROPHOTOMETER

Ultraviolet-visible spectroscopy is considered an important tool in analytical chemistry. In fact, this is one of the most commonly used techniques in clinical as well as chemical laboratories. This tool is used for the qualitative analysis and identification of chemicals. However, its main use is for the quantitative determination of different organic and inorganic compounds in solution. UV spectrophotometer principle follows the Beer-Lambert Law. This law states that whenever a beam of monochromatic light is passed through a solution with an absorbing substance, the decreasing rate of the radiation intensity along with the thickness of the absorbing solution is actually proportional to the concentration of the solution and the incident radiation.

This law is expressed through this equation:

$$A = \log (I^0/I) = ECI$$

A stands for the absorbance, I^0 refers to the intensity of light upon a sample cell, l refers to the intensity of light departing the sample cell, C stands for the concentration of the solute, L stands for the length of the sample cell and E refers to the molar absorptivity. Basing from the Beer-Lambert law, it has been established that the greater the number of the molecules that are capable of absorbing light at a certain wavelength, the greater the extent of the absorption of light.

CHAPTER II - LITERATURE REVIEW:

John Hayman (1985) conducted a research on Datura poisoning- The Angel's Trumpet. A group of seven ate flowers of *Datura arborea* ("The Angel's Trumpet" or "Trumpet Lilies") and suffered severe hallucinations. One member of the group drowned in shallow water while suffering from effects. Although poisoning with related species is common, poisoning with this plant is rare, perhaps due to its terrifying rather than pleasurable hallucinogenic effect. [1]

Philip Salena et.al (2003) reported on the effect of physostigmine and gastric lavage in a Datura stramonium-induced anticholinergic poisoning epidemic. This study examines the impact of the administration of physostigmine and of nasogastric evacuation of Jimsonweed seeds on intensive-care unit (ICU) use and the length of stay in the hospital after Jimsonweed poisoning. Clinical data for this retrospective study were gathered from records of consecutive patients treated for Jimsonweed poisoning from September to November 1997. The use of physostigmine and the successful nasogastric lavage of Jimsonweed seeds did not result in decreased intensive-care use or shorter length of stay in the hospital for Jimsonweed-induced anticholinergic toxicity. [2]

P.A. Steenkamp et.al (2004) conducted analysis on fatal Datura Poisoning identification of Atropine and Scopolamine by High Performance Liquid Chromatography, Photo diode array, mass spectrometry. A forensic method comprising solid phase extraction and HPLC analysis was developed for the detection and confirmation of atropine and scopolamine, the main toxic alkaloids of *Datura stramonium* and *Datura ferox*. The optimised HPLC method was used to analyse three viscera samples of an adult Caucasian male whose death was ascribed to a fatal heart attack. Atropine and scopolamine were detected in the stomach and its contents, which contained Datura seeds. The chemical profile of the seeds found in the stomach contents was similar to those from four geographically different D. ferox plants. [3]

D. Diker et.al (2007) studied the hallucinogenic effects of *Datura stramonium* poisoning. *Datura stramonium* is a hallucinogenic plant that causes serious poisoning.

Consumption of any part of the plant may result in a severe anticholinergic reaction that may lead to toxicity and occasionally cause diagnostic difficulties. We report two patients with coma as a presenting sign of intoxication following intentional *Datura* seed tea ingestion and we review the leading clues for its diagnosis and treatment. [4]

Sourav Khanra, C.R.J. Khess and Naveen Srivastava (2014) reported on Chronic non-fatal Datura abuse in a patient of paranoid schizophrenia. The symptoms that aroused due to the gradually administration of *Datura stramonium* seeds for 3 years. These were done by taking detailed history and mental status examination (MSE) and diagnosis of paranoid schizophrenia and mental and behavioural disorder due to use of hallucinogen were made. [5]

Silvia Jakabovaab et.al (2014) determined of tropane alkaloids atropine and scopolamine by liquid chromatography – mass spectrometry in plant organs of Datura species. Atropine content was more than scopolamine. The analysis of *Datura* extracts revealed significant differences depending on the species, the organ and the sampling period. Liquid chromatography and mass spectrometry was the technique used for the determination of the tropane alkaloid. [6]

Nezihat Rana DISEL et.al (2015) reported a case on *Datura stramonium* poisoning

Datura stramonium, which is also known as Thorn Apple or Jimson Weed, is an alkaloid containing plant that is entirely toxic. The active toxic constituents of the plant are atropine, scopolamine and hyoscyamine. It has been abused worldwide for hundreds of years because of its hallucinogenic properties. Previous reports have shown that herbal medication overdose and accidental food contamination are ways it can cause poisoning. Herein we present a family that had three of its members poisoned after eating a traditional meal "dolma" made of Datura flowers. None had fatal complications and all were discharged healthy. *Datura stramonium* may be used accidentally as a food ingredient. Since its poisonous effects are not known, people should be informed and warned about the effects of this plant. [7]

E. Le Gariff et.al (2016) has conducted a study on the forensic features of fatal Datura poisoning case during a robbery. Datura poisonings have been previously described but remain rare in forensic practice. Here, we present a homicide case involving Datura poisoning, which occurred during a robbery. Toxicological results were obtained by second autopsy performed after one previous autopsy and full body embalmment.. The victim's death was attributed to disordered heart rhythm due to severe anticholinergic syndrome following fatal Datura intoxication. This is a recent case of a rare homicide involving Datura that highlights general information on Datura and discusses forensic interpretation after a previous autopsy and body embalmment. [9]

Temidayo Ogunmoyolea et.al (2019) studied on multiple organ toxicity of Datura Stramonium seed extracts. *Datura stramonium* seed ranks top among major plants commonly abused as drug in Nigeria. The present study therefore sought to unravel the target organs of toxicity as well as underscore the role of extraction solvent in the toxicity of *Datura stramonium* seed. [11]

Chinedu Imoa et.al (2019) conducted an investigation on the effects of ethanolic extracts of leaf, seed and fruit of *Datura metel* on kidney function of male albino rats was investigated in this study. The administration of some of the extracts in comparison with the normal control in histology of the animals show glomerular extrusion and glomerular collapse with resultant increased urinary space, dilated tubules, vacuolations in some epithelial lining of most of the tubules in the medulla and inflammatory cellular infiltration at some peritubular regions. The results showed that some parts of *Datura metel* posed mild negative effects, while some parts could possess nephroprotective potential by regulating the kidney function of male albino rats. [12]

CHAPTER III – AIM AND OBJECTIVE

AIM

To identify the presence of tropane alkaloids of *Datura metel* by Thin Layer Chromatography and UV-VIS Spectrophotometry.

OBJECTIVES

- To identify the tropane alkaloids presence in the food. This helps us to identify that the given food sample is poisoned with Datura metel.
- To evaluate the Retention Factor (R_f) value of the alkaloids and thereby under UV-VIS spectrophotometer to obtain the absorbance.
- The goal is to distinguish the Datura poisoned food.

CHAPTER IV- MATERIALS AND METHODOLOGY

Materials required:

1. Air dried samples of Datura seeds (13 to 14 days has been taking for the complete drying process)
2. Methanol
3. Distilled water
4. Magnetic stirrer
5. Mortar and pestle
6. Electronic blender
7. Measuring cylinders
8. Beakers
9. Glass slides 8x3cm
10. Silica gel
11. Ethanol
12. Oven for activation of the plates
13. Diethyl amine
14. Chloroform
15. Capillary tube
16. Solvent chamber
17. Ruler
18. HPTLC Silica gel 60 F 254 plates 5x10cm
19. Spraying gas N_2
20. Sample solvent – Methanol
21. Syringes
22. Toluene
23. Ethyl acetate
24. Cuvettes

Instruments:

- Electric Oven

- UV chamber
- CAMAG Automatic TLC Sampler 4
- CAMAG Automatic Development Chamber (ADC) 20x10
- CAMAG TLC Scanner 3
- CAMAG Visualizer
- UV Pharmaspec-1700 UV Visible Spectrophotometer, Shimadzu
- Camera

Method:

Extraction:

The air dried *Datura metel* seeds were crushed in fine powder in an electronic blender and passed through 5µm mesh size. *Datura metel* was extracted thrice with 20ml of methanol for 45 minutes in a magnetic stirrer. After mixing they were filtered and vacuum dried at 45ºc. The dried extracts were dissolved in 2ml of methanol and samples of the varying concentrations (5µl of seeds) were spotted for quantification.

Reagent preparation:

Solvent system for TLC: Chloroform: Diethyl amine (90:10)

Solvent system HPTLC: Toluene: Ethyl acetate: Methanol (5:5:1)

TLC

Step 1: The Datura sample was extracted with 100ml of Methanol and stirrer for 45mins in a magnetic stirrer for 45mins after which the sample was kept for 5 hours and concentrated to 10ml.

Step 2: The silica gel plates were made with the help of silica gel, water and glass slides. These plates were activated in Electric Oven.

Step 3: The extract was loaded on the silica plate leaving 1cm space from the bottom.

Step 4: The solvent system was poured into the solvent chamber and the loaded silica plate is placed into the chamber and was kept it for running.

Step 5: After completion of the running of the mobile phase take the plate outside and visualise it in UV chamber.

Step 6: The distance travelled by the solute and solute were measured for the calculation of the R_f values

HPTLC

Step 1: The sample was prepared which is extracted with Methanol

Step 2: Selection of the HPTLC plates, handmade Silica Gel plates for chromatographic layer were used for alkaloids

Step 3: The plates were activated

Step 4: Nitrogen gas was sprayed on the Plates and the sample application was done by using a syringe of 5µl of the sample onto the plate band wise.

Step 5: The chamber was preconditioned by using filter paper for a volume of 100ml

Step 6: 10ml of the solvent system was prepared and poured into the chamber and set it for equilibration for about 5 minutes

Step 7: Insert the plate into the front trough at 80mm of position where the layer was facing the filter paper and the back was resting against the wall of the chamber and develop the plate upto the mark

Step 8: The plate was dried for 240s (4 minutes)

Step 9: The plates were detected under UV light at 254nm and at 366nm

Step 10: The plates were scanned and documented with CAMAG Scanner 3

UV - VIS Spectrophotometry

Step 1: The instrument was powered ON

Step 2: The cuvettes were taken and handled by the edges

Step 3: Load 0.5mL of the sample into the cuvette with the help of a pipette

Step 4: A control solution was prepared known as blank sample that was Methanol. Cuvettes were wiped clean.

Step 5: The wavelength were set and the control sample was placed into the spectrophotometer and the blank sample was set to 0 for calibration, after which it was removed.

Step 6: The sample was placed into the instrument and measured the absorbance and graph was obtained with Absorbance in the x-axis and wavelength in the y axis.

CHAPTER V – OBSERVATIONS AND CALCULTIONS

Observations for TLC:

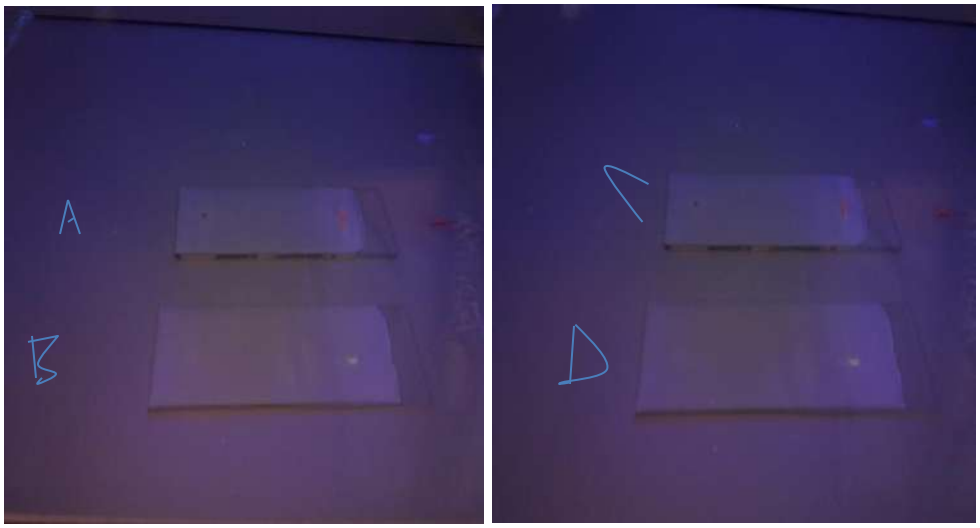
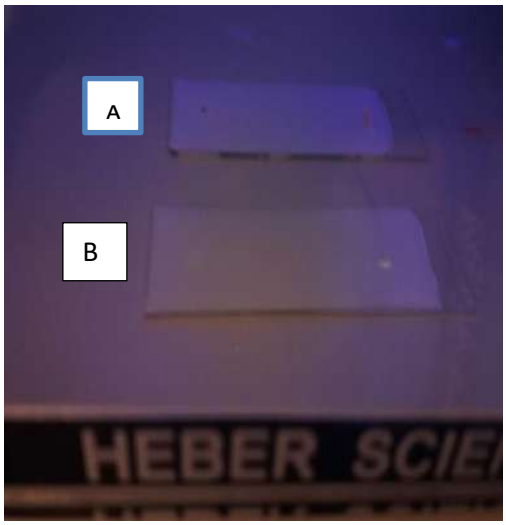


FIGURE 2.1 & FIGURE 2.2: A, B, C and D is marked for Sample 1, Sample 2, Sample 3 and Sample 4 respectively under long wave (352nm)

Sample	Distance travelled by the solute	Distance travelled by the solvent	R_f value	MEAN
Sample 1	4.5	7	0.64	0.645
Sample 2	4.4	7	0.62	
Sample 3	4.6	7	0.65	
Sample 4	4.7	7	0.67	

TABLE 1.1 FIRST OBSERVATIONS OF THE SAMPLES BY TLC



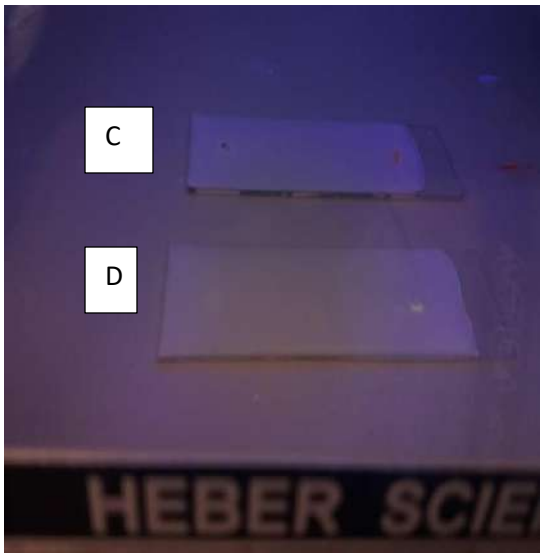


FIGURE 2.3 & FIGURE 2.4: A, B, C and D is marked for Sample 1, Sample 2, Sample 3 and Sample 4 respectively under long wave(352nm)

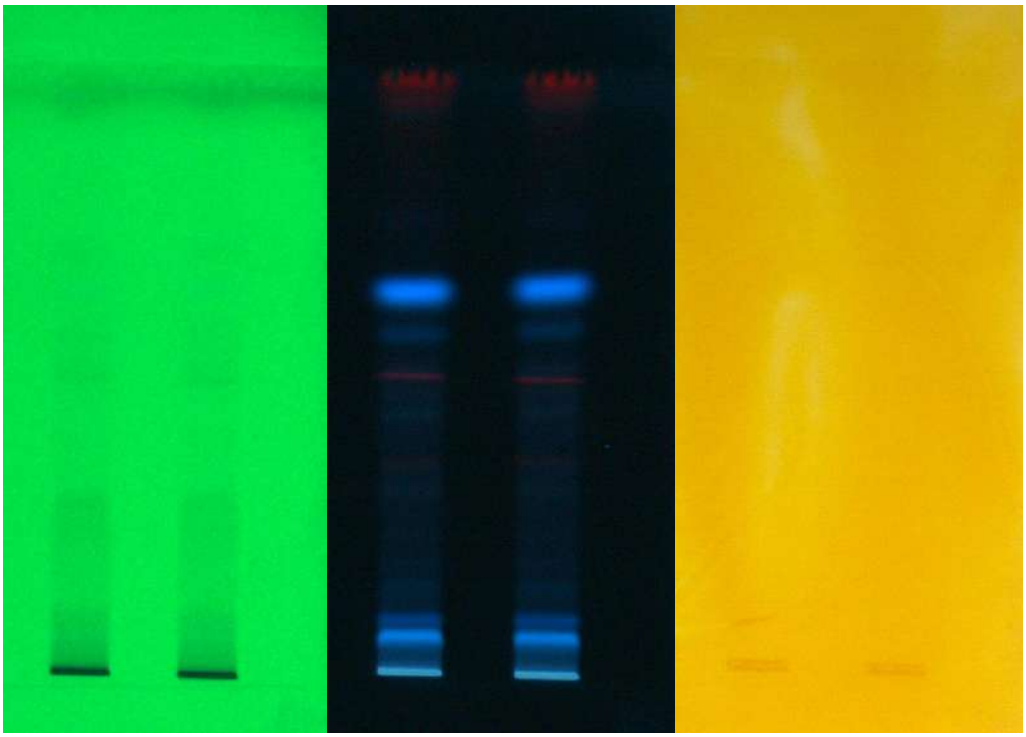
Sample	Distance travelled by the solute	Distance travelled by the solvent	R_f Value	MEAN
Sample 1	4.6	7	0.65	0.64
Sample 2	4.4	7	0.62	
Sample 3	4.6	7	0.65	
Sample 4	4.5	7	0.64	

TABLE 1.2 SECOND OBSERVATIONS OF THE SAMPLES

R_f Values	
Compound	**Chloroform: Diethyl amine**
Atropine/ Hyoscyamine	0.45
Homatropine	0.55
Scopolamine	0.65

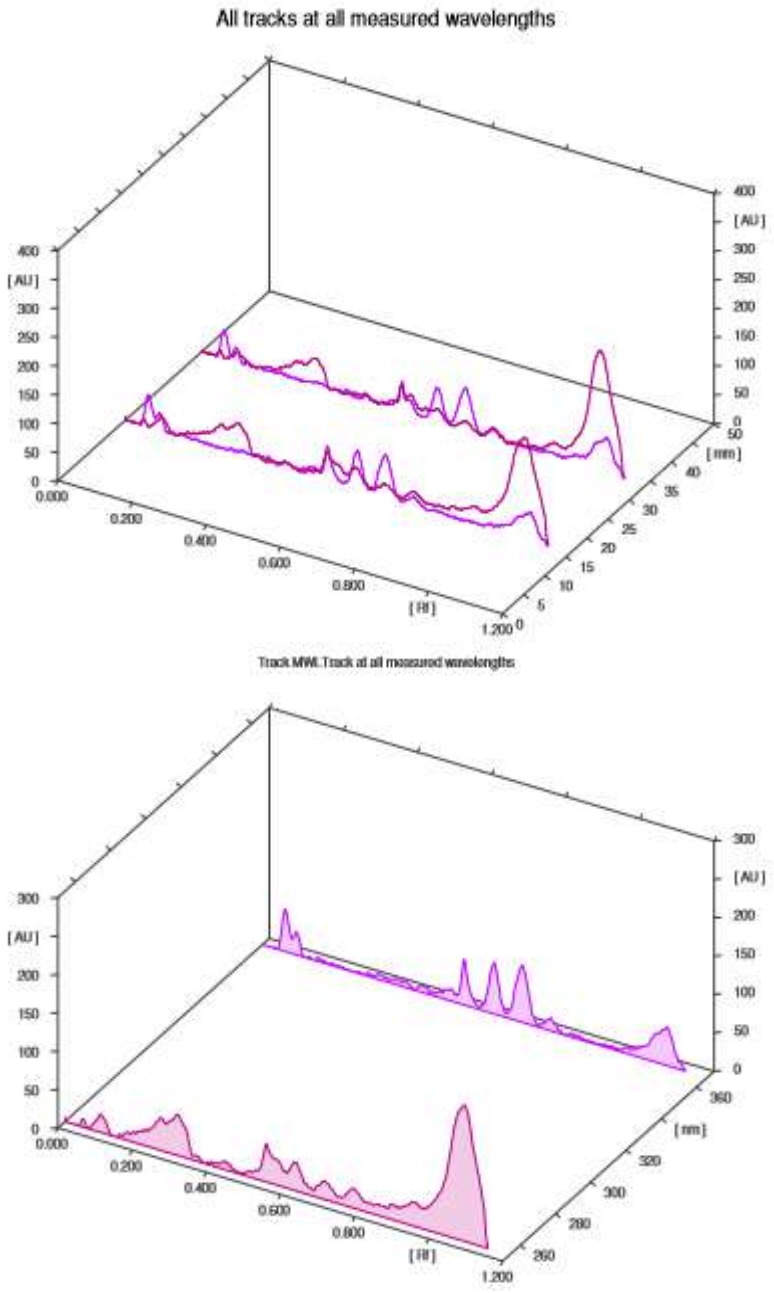
TABLE 1.3[12] REFERENCE R_f VALUES

Observations for HPTLC



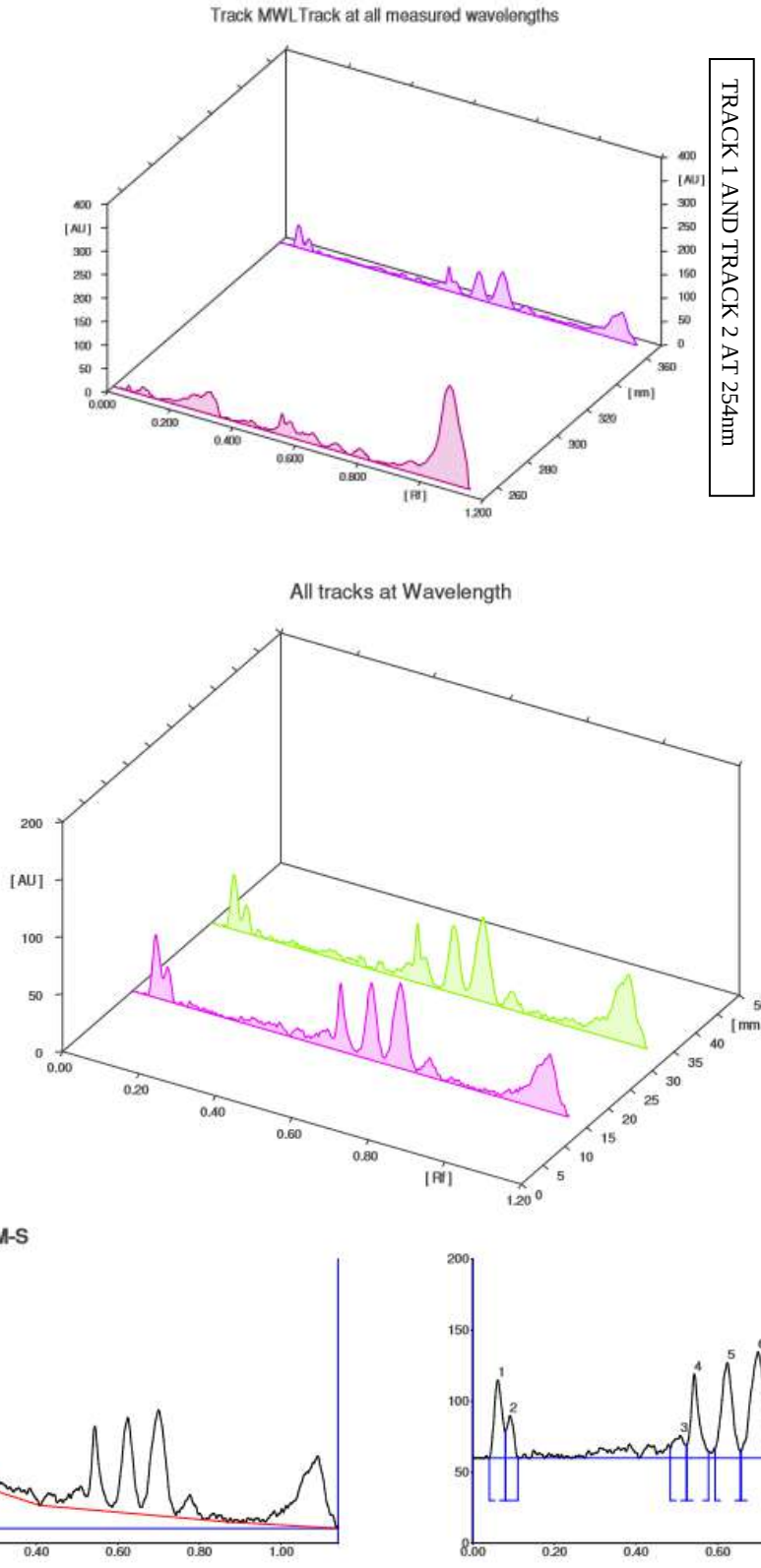
254nm | 366nm | Visible

FIGURE 3 REPRESENTS THE HPTLC PLATES AT 254nm, 366nm AND VISIBLE



GRAPH 1.1: CHROMATOGRAMS AT ALL WAVELENGTHS WITH BOTH THE TRACKS

At 254nm of Wavelength

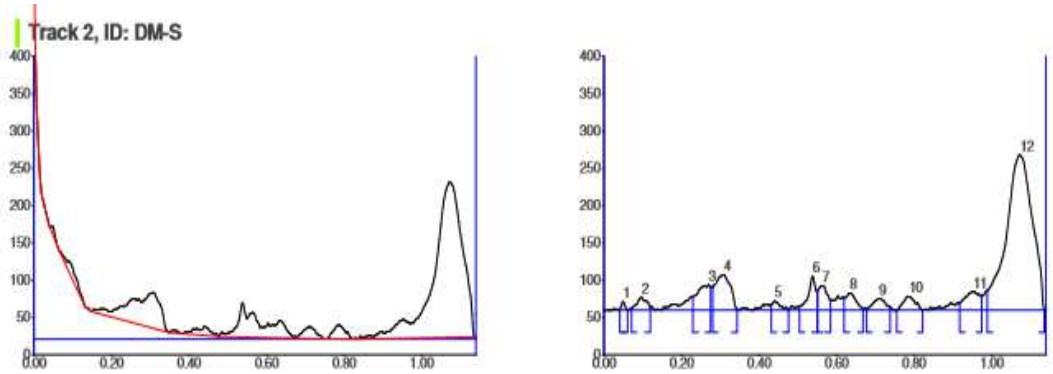


TRACK 1 AND TRACK 2 AT 254nm

GRAPH 1.2: CHROMATOGRAMS OF FIRST TRACKS AT 254nm WAVELENGTH

Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Assigned substances
1	0.03	1.0	0.05	10.8	0.06	0.06	0.4	
2	0.06	0.2	0.10	23.4	0.12	0.12	0.4	
3	0.21	17.5	0.26	42.4	0.27	0.27	36.3	
4	0.28	37.1	0.30	53.2	0.35	0.35	4.9	
5	0.40	3.8	0.43	10.6	0.45	0.45	3.4	Atropine/Hyoscamine
6	0.50	6.8	0.54	49.5	0.59	0.59	20.2	
7	0.60	19.4	0.63	36.7	0.65	0.65	13.0	Scopolamine
8	0.68	15.6	0.70	23.5	0.74	0.74	8.4	
9	0.75	8.9	0.78	25.5	8.31	0.81	12.0	
10	0.90	17.5	0.95	31.4	10.21	0.96	27.2	

TABLE 2.1 REPRESENTS THE DATA OF TRACK 1 CHROMATOGRAM

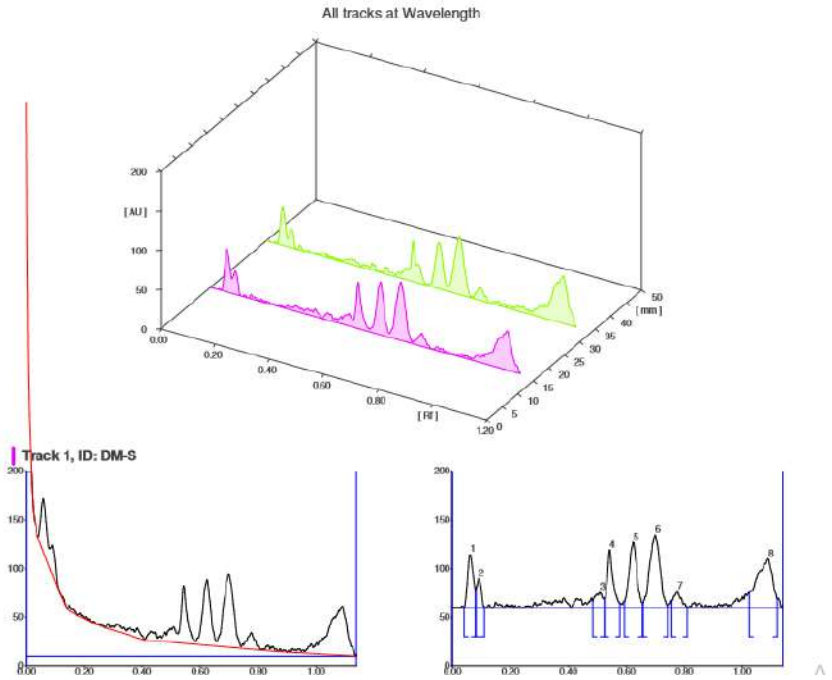


GRAPH 1.3: CHROMATOGRAM OF 2ND TRACK 254nm OF WAVELENGTH

Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Assigned substances
1	0.04	1.1	0.05	11.7	2.36	0.06	0.1	
2	0.07	3.0	0.10	17.4	3.53	0.12	4.4	
3	0.23	17.9	0.27	34.4	6.97	0.28	30.7	
4	0.28	31.1	0.31	47.8	9.69	0.34	1.8	
5	0.43	7.2	0.44	12.8	2.59	0.48	0.0	Atropine/Hyoscamine
6	0.51	5.5	0.54	45.9	9.30	0.55	25.1	
7	0.55	25.9	0.56	33.0	6.69	0.59	13.4	
8	0.62	16.6	0.64	23.1	4.68	0.67	1.7	Scopolamine
9	0.68	1.8	0.71	15.5	3.13	0.74	4.5	
10	0.76	0.0	0.79	19.0	3.85	0.82	0.5	
11	0.92	10.0	0.95	24.8	5.02	0.98	21.2	
12	0.99	26.5	1.08	208.1	42.18	1.14	0.2	

TABLE 2.2 REPRESENTS OF DATA OF THE 2ND TRACK CHROMATOGRAM

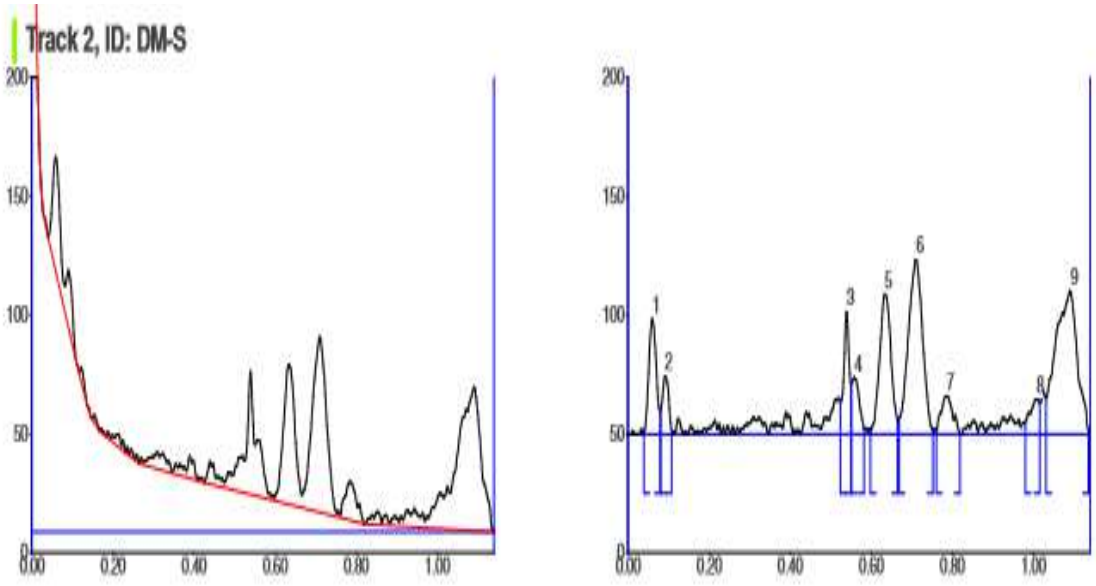
At 366nm of Wavelength



GRAPH 1.4: TRACK 1 AT 366nm OF WAVELENGTH

Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Assigned substances
1	0.04	0.5	0.06	55.0	14.91	0.08	18.4	
2	0.08	19.1	0.09	30.0	8.13	0.11	1.1	
3	0.49	7.0	0.51	16.0	4.33	0.53	9.4	
4	0.53	9.5	0.54	59.2	16.06	0.58	4.6	
5	0.60	6.7	0.63	67.2	18.24	0.66	5.1	Scopolamine
6	0.66	5.1	0.70	74.6	20.23	0.74	1.7	
7	0.76	7.2	0.78	16.7	4.54	0.81	0.2	
8	1.03	15.6	1.09	50.0	13.57	1.12	9.2	

TABLE 2.3 REPRESENTS DATA OF TRACK 1 OF 366nm WAVELENGTH



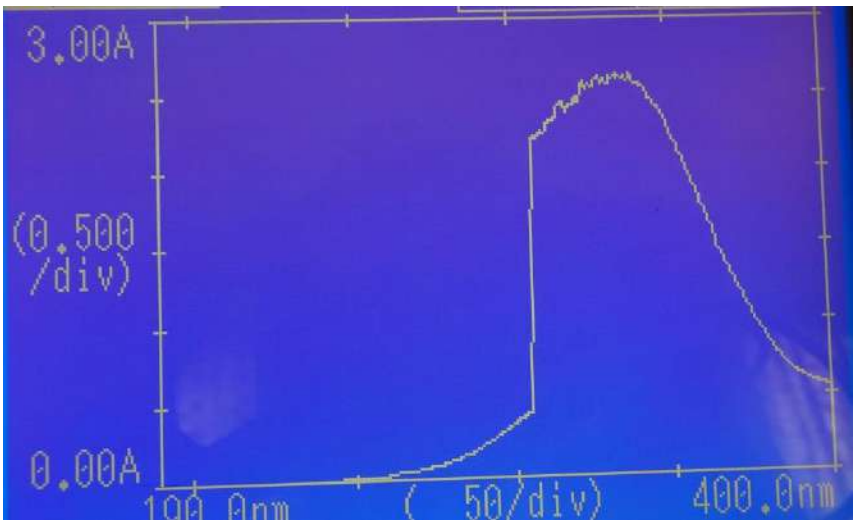
GRAPH 1.5: TRACK 2 AT 366nm OF WAVELENGTH

Peak	Start Rf	Start Height	Max Rf	Max Height	Max%	End Rf	End Height	Assigned Substances
1	0.04	0.0	0.06	48.4	13.0	0.08	9.9	
2	0.08	10.7	0.09	24.5	6.57	0.11	1.0	
3	0.53	13.6	0.54	51.8	13.90	0.55	21.3	
4	0.55	21.5	0.56	23.9	6.41	0.58	2.3	
5	0.60	1.3	0.63	58.7	15.76	0.67	5.3	Scopolamine
6	0.67	7.0	0.71	73.9	19.83	0.75	1.9	
7	0.76	1.1	0.79	16.1	4.31	0.85	0.1	
8	0.98	5.3	1.01	14.9	3.99	1.02	13.5	
9	1.03	15.4	1.09	60.5	16.23	1.14	1.4	

TABLE 2.4 REPRESENTS THE DATA OF TRACK 2 CHROMATOGRAM

Observation for UV-VIS Spectrophotometry:

Spectrum was recorded in the region of 190 - 400 nm in the UV region.



GRAPH 2: REPRESENTS THE SPECTRUM OBTAINED FROM UV-VIS SPECTROPHOTOMETER BY THE ANALYSIS OF DATURA METEL

CHAPTER VI- RESULT AND CONCLUSION

RESULT:

By TLC method of identification the obtained two R_f values are 0.645 and 0.64 which is identified as scopolamine from reference. Through HPTLC method of analysis at various wavelengths such as 254nm and 366nm the R_f values for Atropine, Hyoscyamine and Scopolamine were obtained.

By UV-VIS Spectrophotometer Λ_{max} from 190nm to 400nm is obtained. From the previous studies on Datura metel and the alkaloids atropine gives its absorbance at 254nm [15] of wavelength.

CONCLUSION:

The Tropane Alkaloids of Datura metel were identified from the sample by Thin Layer Chromatography, High Performance Thin Layer Chromatography and UV-VIS Spectrophotometry. Keeping these values as reference it can be used to compare the analysed values of Datura poisoned food and beverages and even viscera samples. By the end of this project work extraction of the tropane alkaloids of Datura metel was obtained in a successful manner so as to perform the experiment on the identification of alkaloids of Datura metel by thin layer chromatography and UV-VIS spectrophotometer. In future research can be conducted on the same by High Performance Liquid Chromatography, Gas Chromatography, Mass Spectroscopy and FTIR.

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