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TO EVALUATE THE CHEMICAL CONSTITUENTS OF SHODITHA (PURIFIED) AND ASHODHITHA (RAW) *DATURA METEL LINN* ROOT – A ANALYTICAL STUDY

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Keywords:

Datura metel Linn., Root, Visha, Physcio-chemical, Phyto-chemical analysis

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ABSTRACT: *Datura* (*Datura metel Linn*.) is a herbaceous leafy annual and short-lived plant of the Solanaceae family. It's commonly known as Angel's trumpet, Devils apple, Jimson weed, stinkweed, and Thorn apple. The traditional system of medicine enlightened the importance of *Datura metel Linn*. of its great medicinal value. The roots, leaves and flowering tops have been used in India for their narcotic and antispasmodic properties in the treatment of numerous ailments and conditions. The juice of fresh leaves is used to relieve the painful swellings. The seeds and root possess anti-diarrheal and are used to treat insanity and fever. All parts of *Datura* are poisonous in nature, and it's mentioned under sthavara visha category by Ayurveda. In the present study, roots of *Datura*, which are less toxic compared to the other parts of the plant, were studied both in raw and purified form. AS the drug, in general, was considered poisonous, purification was done with Ayurveda procedure (Shodhana Samskara) to reduce the toxicity of the noxious drug for its chemical profile and pharmacological potential through physciochemical and phyto-chemical analysis.

INTRODUCTION: *Datura* (*Datura metel Linn*.) is a herbaceous leafy annual and short-lived plant of the Solanaceae family with perennials that can grow up to two meters of height found at all tropical regions of India mainland [1]. It's commonly known as Angel's trumpet, Devils apple, Jimson weed, stinkweed, and Thorn apple. The name *Datura* comes from the early Sanskrit terminologies Dustura or dahatura.

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The plant is considered poisonous, among which the seeds are considered the more toxic compared to other parts of the plant. Even though toxic, *Datura* is used as a phytomedicine to treat various diseases. The whole plant, particularly the leaves and seeds, is used as an anesthetic, antispasmodic, anodyne, anti-asthmatic, anti-tussive, bronchodilator, and as hallucinogenic [2].

It is also used to manage skin diseases, ulcers, burns, rheumatic pains, hemorrhoids, diarrhea, epilepsy, hysteria and insanity. It's even used to calm the cough and treat laryngitis and trachitis [3]. A variety of phytochemicals have been found in *Datura metel Linn*. such as alkaloids, flavonoids, phenols, tannins, saponins and sterols [4]. The whole plant of Datturametel Linn. contain Scopolamine

(Hyoscine) and. Atropine increases gradually with the progress of developmental growth and is most pronounced when the plant is at the end of its reproductive stage. The Scopolamine accumulation is highest in the root after 16 weeks [4]. The root contains a higher amount of atropine as compared to the other parts. The areal part usually accumulated relatively higher amounts of Scopolamine and relatively lower amounts of atropine.

The total alkaloid contains 0.426%, which is mainly atropine. The seeds contain 0.426% of alkaloids which is mainly hyoscyamine. The roots contain 0.35% hyoscyamine [4]. *Datura* contains dangerous levels of tropane alkaloids which are highly poisonous and is fatal if ingested; it can produce symptoms like dryness of mouth, nausea, vomiting, dysphasia dysarthria, diplopia, delirium, hallucination, dry and hot skin (due to inhibition of sweat secretion) and red (due to the dilatation of cutaneous blood vessels) skin especially in the face/ chest, drowsiness later it can lead to coma than death [5].

Ayurveda discusses *Datura* (*Datura metel Linn*.) under the Upavisha category of plants [6] and as specified, any potent poison can be utilized in treatment as an excellent medicine if used judiciously, on the contrary. This potent medicine can act as a potent poison if used injudiciously [7]. In Ayurvedic formulations, the leaf, seed, stem and even the plant as such, including its roots, are widely used for its ant cholinergic and delirium properties. AS *Datura* is poisonous before using detoxification of the plant part must be done, here Dola yantra swedana method of shodhana samskara is used with godugdha (cow milk) as shodhana dravya [8] Shodhanasamskara is done with shodhana dravya which is separately mentioned in Ayurveda for each. In the present study, Ashodhitha *Datura* moola (Raw *Datura metel Linn*. root) and Shodhitha *Datura* moola (detoxified *Datura metel Linn*. root) was used for the study.

MATERIALS AND METHODS:

Drug Collection and Preparation: Raw *Datura* (*Datura metel Linn*.) roots were collected from the Ernakulam District of Kerala state in March. It was dried in sunlight for 4 days; once the root was dried, it was subjected to shodhana samskara.

Shodhana Samskara: Specific shodhana method for *Datura* root was not available; the method of Dolayantra swedana explained for detoxification of *Datura*beeja (seed) was implemented using Godugdha (cow milk) as shodhana dravya.

Material: *Datura* root, godughdha (cow milk) both in the required quantity, earthen pot, cotton cloth, gas stove, and cylinder.

Shodhana of Drug:

- Shodhana of the drug was done by Dola yantra swedana with godughdha
- The dried drug (*Datura*Moola) was chopped into small pieces so that a pottali can be made for swedana purposes.
- Then a pottali was made using a medium-thick cotton cloth (thickness must be in such a way that the cloth must not tear, but it must help in shodhana of the drug).
- Then a potali was dipped into DolaYantra filled with milk (quantity sufficient so that pottali completely dips in milk).
- Swedana was done for 3 h.
- After kept under sunlight for drying.
- After the drug got completely dried, it was powdered using a pulverizer, and it was made into nice powder (maximum nice).
- Then it was packed in airtight packets according to requirement.

Physcio-Chemical Analysis and Phyto-Chemical Analysis: Analytical study is the application of a process or a series of processes to identify and/or quantify a substance, the components of a solution or mixture, or the determination of the structures of chemical compounds.

Samples Taken:

- Shodita *Datura* moolaChurna.
- *Ashodita Datura* moolaChurna.

Methods Opted For Analysis:

- Loss on drying,
- Ash value
- Acid insoluble ash

- Alcohol soluble extractive
- Water soluble extract
- HPTLC
- Preliminary phytochemical tests

Methodology [9]:

Loss on Drying at 105 °C: 10 g of sample was placed in tared evaporating dish. It was dried at 105 °C for 5 h in hot air oven and weighed. The drying was continued until the difference between two successive weights was not more than 0.01 after cooling in a desiccator. Percentage of moisture was calculated with reference to weight of the sample.

Total Ash: 2 g of sample was incinerated in a tared platinum crucible at a temperature not exceeding 450 °C until carbon-free ash is obtained. The percentage of ash was calculated with reference to the weight of the sample.

Acid Insoluble Ash: To the crucible containing total ash, add 25 ml of dilute HCl. Collect the insoluble matter on ashless filter paper (Whatmann 41) and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate, and ignite constant weight. Allow the residue to cool in suitable desiccator for 30 min and weigh without delay. Calculate the content of acid insoluble ash with reference to the air dried drug.

Alcohol Soluble Extractive: Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled alcohol (approximately 95%). Shake occasionally for 6 h. Allow to stand for 18 h. Filter rapidly taking care not to lose any solvent. Pipette out 25 ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105 °C for 6 h, cool in a desiccator for 30 min and weigh. Calculate the percentage of Alcohol extractable matter of the sample. Repeat the experiment twice, and take the average value.

Water Soluble Extractive: Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled water, shake occasionally for 6 h. Allow to stand for 18 h. Filter rapidly taking care not to lose any solvent. Pipette out 25 ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105 °C for 6 h. Cool in a desiccator and weigh. Repeat the experiment twice. Take the average value.

HPTLC: 1 g of the powdered drug was extracted with 10 ml of alcohol. 5, 10 and 20 µl of the above extract was applied on a precoated silica gel F254 on aluminum plates to a bandwidth of 8 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate: Formic acid (6: 0.5: 0.1). The developed plates were visualized in UV 254, 366, and after derivatization with vanillin-sulphuric acid and scanned under UV 254 and 366. Rf, colour of the spots, and densitometric scan were recorded.

Preliminary Phytochemical Tests: are used to detect various organic functional groups, which indicates the type of phytochemicals present in the plant. These tests indicate the presence of a different class of constituents present in the extract. Tests were performed as per the methodology mentioned by Harborne JB.

The following tests have been carried out for Alcoholic and Aqueous extracts.

Tests for Alkaloids

Dragendroff's Test: To a few mg of extract dissolved in alcohol, a few drops of acetic acid and ragendroff'sreagent were added and shaken well. An orange-red precipitate formed indicates the presence of alkaloids.

Wagners's Test: To a few mg of extract dissolved in acetic acid, a few drops of Wagner's reagent was added. A reddish-brown precipitate formed indicates the presence of alkaloids.

Mayer's Test: To a few mg of extract dissolved in acetic acid, a few drops of Mayer's reagent was added. A dull white precipitate formed indicates the presence of alkaloids.

Hager's Test: To a few mg of extract dissolved in acetic acid, 3 ml of Hager's reagent was added, the formation of a yellow precipitate indicates the presence of alkaloids.

Tests For Carbohydrates

Molisch's Test: To the extract, 1 ml of α-naphthol solution and conc. sulphuric acid were added

along the sides of test tube. Violet colour formed at the junction of the two liquids indicates the presence of carbohydrates.

Fehling's Test: A few mg of extract was mixed with equal quantities of Fehling's solution A and B.

The mixture was warmed on a water bath. The formation of a brick-red precipitate indicates the presence of carbohydrates.

Benedict's Test: To 5 ml of Benedict's reagent, a few mg of extract was added and boiled for two minutes, and cooled.

The formation of a red precipitate indicates the presence of carbohydrates.

Anthrone-Sulphuric Acid Test: A few mg of the extract was mixed with an equal quantity of anthrone and treated with two drops of conc. sulphuric acid. It was then heated gently on a water bath. The dark green colour formed indicates the presence of sugar/glycoside.

Test for Steroids

Libermann-Burchardtest: To the extract was dissolved in chloroform, 1 ml of acetic acid and 1 ml of acetic anhydride were added, then heated on a water bath and cooled.

Few drops of conc. Sulphuric acid was added along the sides of the test tube. The appearance of bluish-green colour indicates the presence of steroids.

Salkowski Test: The extract was dissolved in chloroform and an equal volume of conc. Sulphuric acid was added.

The formation of bluish-red to cherry red colour in the chloroform layer and green fluorescence in the acid layer indicates the presence of steroids.

Test for Saponins: To a few mg of extract, distilled water was added and shaken. Stable froth formation indicates the presence of saponins.

Test for Tannins: To the extract, a few drops of dilute solution of ferric chloride was added, formation of dark blue colour shows the presence of tannins.

Test for Flavonoids:

Shinoda's Ttest: To the extract in alcohol, a few magnesium turnings and few drops of conc. hydrochloric acid were added and heated on a water bath. The formation of red to pink colour indicates the presence of flavonoids.

Test for Phenol: To the extract in alcohol, added two drops of alcoholic ferric chloride. The formation of blue to blue black indicates the presence of phenol.

Test for Coumarins: To the extract in alcohol,a few drops of 2 N sodium hydroxide solution were added. Dark yellow colour formation indicates the presence of coumarins.

Test for Triterpenoids: The extract was warmed with tin bits and few drops of thionyl chloride. Formation of pink colour indicates the presence of triterpenoids.

Test for Carboxylic Acid: Extract dissolved in water is treated with sodium bicarbonate. Brisk effervescence indicates the presence of carboxylic acid.

Tests for Aminoacids: Extract dissolved in alcohol treated with few drops of ninhydrin solution. Violet colour indicates the presence of amino acids.

TLC/HPTLC Finger Print Studies [10]: Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) were performed as per the standard methods

Chromatographic Conditions: CAMAG HPTLC instrument, sample applicator - CAMAG Linomat - IV applicator with N2 gas flow, photo documentation system - Digi store - 2 documentation system with Win Cats & video scan

software, scanner - CAMAG HPTLC scanner - 3 (030618), Win Cats - IV, development chamber – CAMAG TLC 10 × 10, 10 × 20 twin trough linear development chamber, quantity

applied - 5, 10 µl of extracts, stationary phase - Aluminium plate pre-coated with silica gel 60 F254

(E. Merck), plate thickness - 0.2 mm, scanning wavelength - 254 nm, laboratory condition - 26 ± 5 °C and 53 % relative humidity.

RESULTS:

TABLE 1: PHYSCIO CHEMICAL PARAMETERS RESULTS N = 3% W/W

S. no	PARAMETERS	SHODITA	ASHODITA
1	Total Ash	3.7	11.15
2	Acid insoluble ash	0.55	2.55
3	Loss on drying	7.84	9.08
4	Water soluble extractive	4.93	12.66
5	Alcohol soluble extractive	6.64	2.39

Phytochemical Parameters

TABLE 2.1: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF SHODITHA AND *ASHODITHA DATURA* MOOLA

Tests	Color if positive	Shoditha	Ashoditha
	Alkaloid		
Dragendroff's test	Orange-red precipitate	Orange solution, no precipitate	Orange solution, no precipitate
Wagner's test	Reddish-brown precipitate	Reddish-brown solution	Reddish-brown solution
Mayer's test	Dull white precipitate	Reddish-brown solution	Reddish-brown solution
Hager's test	Green yellowish turbid	Yellow colour	Yellow colour
	Carbohydrate		
Molisch's test	Violet ring	Violet ring	Violet ring
Fehling's test	Brick red precipitate	Brick red precipitate	Brick red precipitate
Benedict's test	Red precipitate	Red precipitate	Red precipitate
Anthronesulphuric acid test	Dark green	Dark green	Dark green
	steroids		
Liebermann-Buchard test	Dark green solution	Green colour	Green colour
Salkowski test	Bluish red to cherry red	Cherry red colour	Cherry red colour
	Saponins		
With $NaHCO_3$	Stable froth	No froth	No froth
On shaking with water	Frothing	No froth	No froth
	Tannins		
With $FeCl_3$	Dark blue or green color	No blue /green colour	No blue /green colour
	Flavinoids		
Shinoda's test	Red to pink	No red or pink colour	No red or pink colour
	Phenol		
with $FeCl_3$	Blue to blue black, green	No blue or black colour	No blue or black colour
	Coumarins		
With 2N NaOH	Dark yellow	White precipitate	White precipitate
	Triterpenoids		
Liebermann-Buchard test	Pink	Light brown solution	Dark brown colour
Tin and thionyl chloride test	Pink	Green with yellow solution	Light yellow
	Resins / Wax		
With distilled water, acetone	Tubidity	No turbidity	No turbidity
	Quinine		
0.5 % sodium hydroxide	Dark pink, purple, red	White precipitate	Brown colour
	Amino acids		
With ninhydrin solution	Violet colour	No violet colour	No violet colour

DISCUSSION: Datura (Daturametel Linn.) is a drug mentioned under upavisha category according to Ayurveda ie its poisonous drug as a whole, still it's used in medicinal formulations after Shodhana or purification procedure. The Physcio chemical study of both Shodita and *Ashodita Datura* mula was done and it was observed thatthe Ash value which is useful in determining authenticity and purity of the sample and also these values are important qualitative standards were, totalash value, acid insoluble ash, was found to be 11.15%, 2.55% in Ashoditha and 3.7 %, 0.55% in shoditha respectively. This percentage clearly indicates that the root is good for drug action and effects.

The Water-soluble extractive value plays an important role in the evaluation of crude drugs. The

less extractive value indicates the addition of exhausted material, adulteration, or incorrect processing during drying or storage.

'The alcohol-soluble extractive value was also indicative for the same purpose as the water-soluble extractive value.

The water-soluble extractive value proved to be higher than alcohol soluble extractive value.

It was found to be 12.66%, 4.93% ashodhita, and shodhitha respectively in water-soluble extractive and 2.39% 6.64% Ashoditha and shodithain alcohol soluble extractive respectively.

TABLE 2.2: RESULTS OF PRELIMINARY PHYTOCHEMICAL TESTS

Test	Shoditha	Ashoditha
Alkaloid	+	+
Amino Acid	-	-
Coumarin	-	-
Flavanoid	-	-
Carbohydrate/glycoside	+	+
Steroid	+	+
Phenol	-	-
Tannin	-	-
Terpenoid	-	-
Resins/wax	-	-
Saponins	-	-
Quinone	-	-

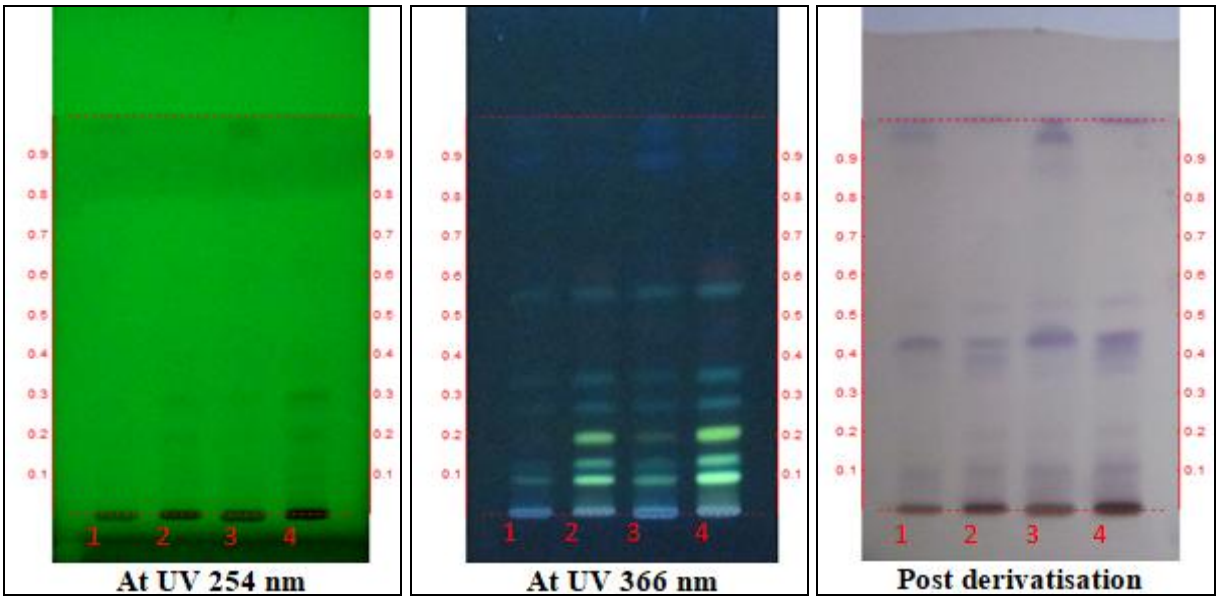

FIG. 1: TLC PHOTO DOCUMENTATION OF ALCOHOL EXTRACT OF SHODITHA AND *ASHODITHA DATTURA* MOOLA

Track 1: Alcohol extract of ShodithaD attura Moola 5 µl
Track 2: Alcohol extract of Ashoditha Dattura Moola 5 µl
Track 3: Alcohol extract of Shoditha Dattura Moola 10 µl
Track 4: Alcohol extract of Ashoditha Dattura Moola 10 µl

Solvent System - Toluene: Ethyl Acetate: Formic Acid (6: 0.5: 0.1)

TABLE 3: RF VALUES OF ALCOHOL EXTRACT OF SHODITHA AND *ASHODITHA DATURA* MOOLA

Solvent system	At UV 254nm		At UV 366nm		Post derivatisation	
	Shoditha	*Ashoditha*	*Shoditha*	*Ashoditha*	*Shoditha*	*Ashoditha*
	-	0.03 L Green	-	-	0.03 Brown	0.03 Brown
	0.08 L Green	0.08 L Green	0.08 F Yellow	0.08 F Yellow	-	-
	-	-	-	-	0.10 Brown	0.10 Brown
	-	-	0.13 F Green	0.13 F Green	-	0.13 L Brown
	0.18 L Green	-	0.18 F Green	-	0.18 Brown	-
	-	0.20 L Green	-	0.20 F Yellow	-	0.20 L Brown
	0.29 L Green	0.29 L Green	0.29 F L Green	0.29 F L Green	-	-
Toluene : Ethyl	-	-	0.35 F L Green	0.35 F L Green	0.35 L Brown	0.35 Violet
acetate: Formic	-	-	-	0.41F Pink	-	0.41 Violet

acid (6: 0.5:	-	-	-	-	0.44 Violet	0.44 Violet
0.1)	-	-	-	0.52 F Pink	0.52 Violet	0.52 Violet
	-	-	0.57 F L Green	0.57 F L Green	-	-
	-	-	0.61 F Pink	0.61 F Pink	-	-
	-	-	0.67F Blue	0.67 F Blue	-	-
	-	-	-	-	0.87 Violet	-
	-	-	0.90 F Blue	0.90 F Blue	-	-
	0.97 L Green	-	0.97 F Blue	0.97 F Blue	0.97 Violet	-
		-	-	-	0.99 Violet	0.99 Violet

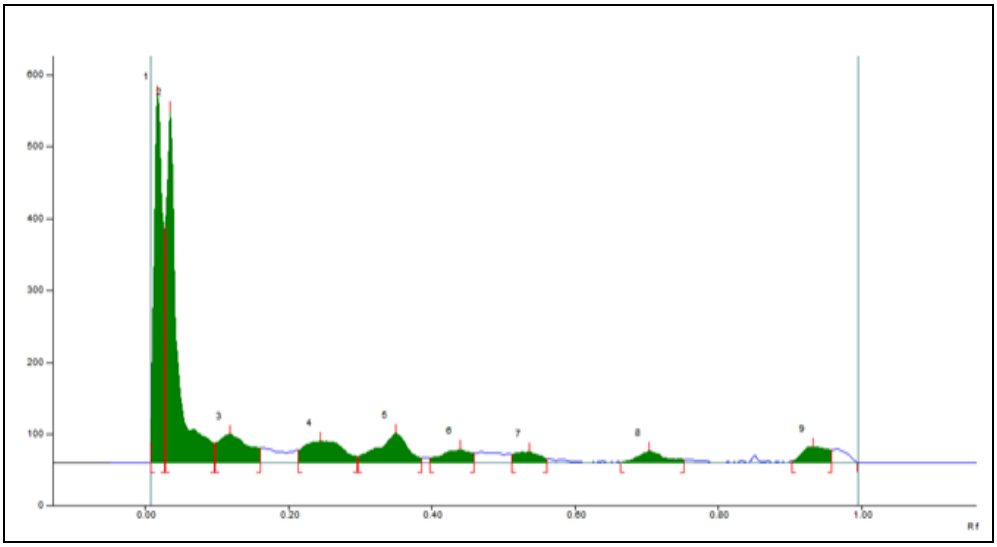

FIG. 2: HPTLC DENSITOMETRIC SCAN OF ALCOHOL EXTRACT OF SHODITHA DATURAMOOLA (10 µL) AT 254 NM

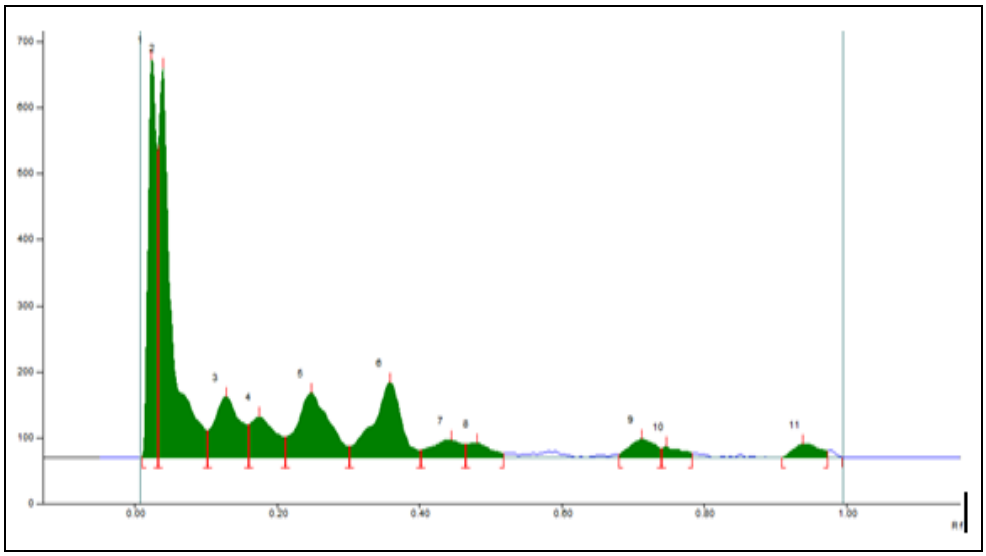

FIG. 3: HPTLC DENSITOMETRIC SCAN OF ALCOHOL EXTRACT OF ASHODITHA DATTURA MOOLA (10 µL) AT 254 NM

This shows that the constituents of the drug are more extracted and soluble in water as compared to alcohol in shoditha Daturamula and its vice versa in *Ashodita Datura* Mulaloss on drying was 7.84% in shoditha and 9.08% in Ashoditha datura mula (root) respectively its influenced by the shodhana dravya (Godugdha) used for the detoxification purpose. The preliminary phytochemical studies in the test drugs revealed alkaloids, Carbohydrate, glycoside & Steroidsin both Ashodhita Datura Mula and Shodhita Daturamula in Godugdha Shodhita. In Shodhita datura beeja, Steroids were found to be present. The presence of alkaloids in both Datura samples *i.e* ashodhita and shodhita samples indicates that alkaloids have not been completely removed but some percent of alkaloids

reduction might have occurred in the Daturamula (Daturametellinn. root) after the detoxification procedure which may be responsible for the improved pharmacological action. The TLC profile of ethanol extract of Shoditha and Ashoditha Datura moola is shown in **Fig. 1.** The R_f values of various spots in the TLC profile are given in the **Table 3.** At 254 nm Shoditha and Ashoditha Datura Moola 4 spots each were observed, at 366nm Shoditha Datura moola showed 10 spots, Ashoditha Datura moola showed 12 spots, and the plate when derivatized showed 9 spots. HPTLC Densitometry scan of alcohol extract of Shoditha and *Ashoditha Datura* Moola (10 µl) at 254 nm, alcohol extract of Shoditha showed 9 peaks **Fig. 2.** of which 2 were major peaks at $_{Rf}$ 0.10, 0.3, whereas Ashoditha showed 11 peaks **Fig. 3.** of which 1 was a major peak at R_f 0.3, rest all are smaller peaks, at 366 nm alcohol extract of Shoditha showed 8 peaks **Fig. 4.** of which 1 major peak at 0.3 $_{Rf}$ and others at 0.09, 0.60 R_f were moderately small and rest were small peaks and in Ashoditha 10 peaks were observed in which 1 major peak **Fig. 5.** at 10 R_f., 3 moderate peaks at 0.01 R_f, 0.15 R_f. and 0.21 R_f respectively, all others were smaller peaks

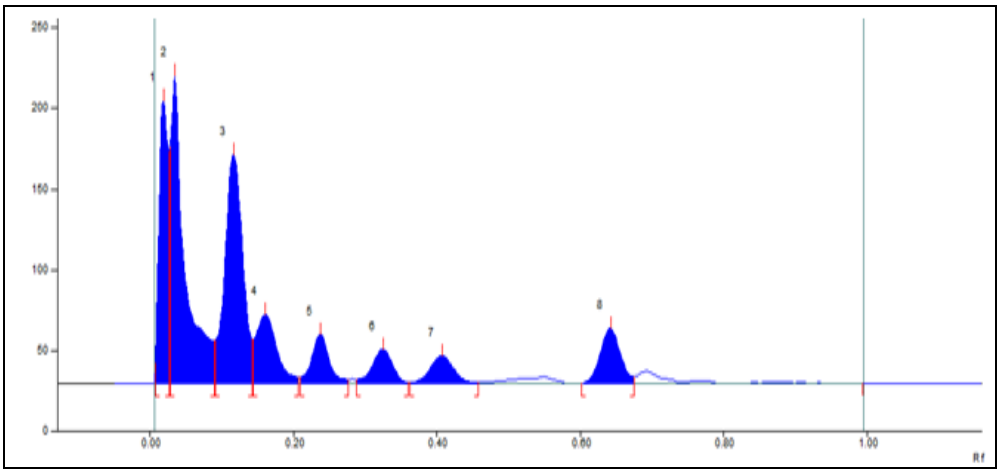

FIG. 4: HPTLC DENSITOMETRY SCAN OF ALCOHOL EXTRACT OF SHODITHA DATURA MOOLA (10 µL) AT 366

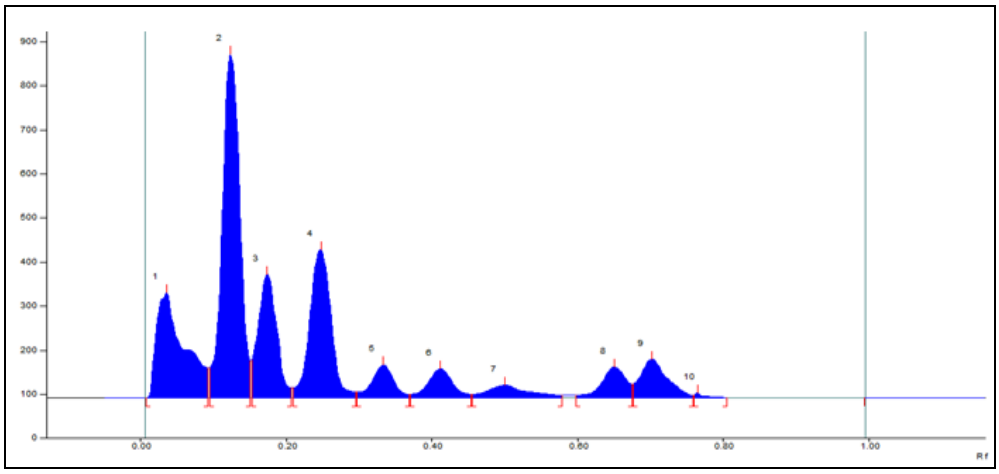

FIG. 5: HPTLC DENSITOMETRY SCAN OF ALCOHOL EXTRACT OF ASHODITHA DATURA MOOLA (10 µL) AT 366 NM

CONCLUSION: The various analytical standards evaluated provide useful data in the standardization and quality control of the drug. The results of preliminary Phytochemical screening of *Datura metel Linn*. root revealed the presence of alkaloids, flavonoids, saponins glycosides. Herbal medicines are composed of many constituents and are therefore capable of variation. Hence, it is very important to get reliable chromatography fingerprints that represent herbal medicine's pharmacologically active and chemically characteristic components. The obtained TLC/HPTLC fingerprinting profile of various extracts of *Datura metel Linn*. root was used as a quality control tool

and will play a major role in herbal drug standardization in the proper identification of the plant.

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