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PHARMACOGNOSTICAL AND PHYSICOCHEMICAL STUDIES ON *ADHATODA VASICA NEES*. SEED

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Keywords:

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ABSTRACT: *Adhatoda vasica Nees.* has been used in India for more than 2000 years. The drug contains leaf, stem, flower, fruit and seeds. The fruit which holds the most potentialof the herb is a small capsule with four seeds. No reports are available for the pharmacognostical study of the seed, hence the present study was undertaken to investigate the macroscopic, microscopic, powder microscopic, physicochemical, phytochemical analysis, TLC and HPTLC profile. The drug was mounted on FAA solution and sections were taken in rotary microtome, stained with toluidine blue; histochemical tests were observed. Loss on drying, total ash, water soluble and acid insoluble ash, water and alcohol soluble extractive were estimated as per WHO method. TLC/HPTLC studies were based on many trials to fix the better solvent system. The testa comprises outer sclerotesta of 40mm thick and inner sarcotesta. The parenchymatous zone is thin along the lateral part of the seed and it becomes wider and many layered at the chalazal end. The radicle is circular in sectional view which is 550 µm in diameter. Sarcotesta, cotyledons, oil bodies, starch grain and the sclerotesta which appears amoeboid in outline were observed in the powder microscopy. 3 spots under UV 254 nm, 4 spots each under 366 nm and after derivatization were observed in the TLC. 8 peaks at 254 nm, 2 peaks at 366 nm and 7 peaks at 540 nm were resolved in HPTLC. The results will be useful to establish the identification, authentication and practical application of the seed as an herbal drug.

INTRODUCTION: *Adhatoda vasica Nees.* an evergreen shrub of 1-3 feet **(Figure 1)** in height belongs to the family Acanthaceae. It has been used in India for more than 2000 years. Commonly known as Malabar nut tree. Synonyms: Aadaathodai (Tamil), Vasaka (Sanskrit), adampaka (Telugu), adusogae (Kannada). Leaves are opposite and large, stem herbaceous on top and woody below. Flower spikes or panicles and white or purple in colour. Capsular fruit with four seeds [1, 2]. The drug contains leaf, stem, flower, fruit and seeds [3]. Pharmacognostical study, the preliminary step in standardization gives valuable information regarding the morphological, microscopical, physical characteristics of the crude drugs and consequently gives the scientific information as regards to the purity and quality of the drugs. The observations of the pharmacognostical studies done on many important drugs have been incorporated in various pharmacopoeias [4]. Literature review has revealed that no pharmacognostical studies have been carried out on the seeds of *A. vasica*, hence the present study was undertaken.

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FIGURE 1: HABITAT OF *ADHATODA VASICA NEES*

MATERIALS AND METHODS:

Plant Materials

A. vasica seeds were collected from the Ayurveda Regional Research Institute, Joginder Nagar, Mandi, Himachal Pradesh, India. It was identified, authenticated and a voucher specimen NIS/MB/59/2012) was deposited in the Department of Medicinal Botany, National Institute of Siddha, Chennai.

Macroscopic and microscopic studies

The seeds were fixed in FAA Formalin - 5 ml + Acetic acid - 5 ml + 70% Ethyl alcohol – 90 ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol[5].Infiltration of the specimens was carried by gradual addition of paraffin wax melting point 58-60^{0}C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was by customary procedure [6]. The sections were stained with toluidine blue since it is a polychromatic stain [7].

Photomicrographs

Photographs of different magnifications were taken with Nikon labphoto 2 microscopic unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard anatomy books [8].

Physico-chemical studies

All the physico-chemical parameters were carried out as per the methods mentioned in standard books[9].

Preliminary phytochemical screening

All the preliminary phytochemical tests were carried out as per the methods mentioned in standard organic books [10, 11].

Preparation of extract for TLC/HPTLC

4g of the drug was first refluxed with 100 ml of hexane and filtered to remove the fatty material. Repeated the process with another 100 ml of hexane. Then the residue was soaked overnight in chloroform. Boiled on a water bath for 10 minutes, filtered and concentrated to 10 ml.

Solvent system

The suitable solvent system was achieved by trial and error method. The solvent system of Toluene: Ethyl acetate 10:1.5, v/v) showed a better resolution than the other solvent systems attempted. This solvent system was used for developing the extract on the TLC plate.

Visualizing reagent

The vanillin-sulphuric acid reagent was chosen as visualizing reagent one gram vanillin dissolved in the mixture of ethanol: sulphuric acid in the ratio 95:5) since it gives colour with most of the categories of secondary metabolites.

Instrument

The twin trough chamber CAMAG) was used for developing the TLC plate. For applying the extract, Linomat IV CAMAG, Muttenz, Switzerland) applicator was used. The TLC plate is made up

aluminium sheet precoated with silica gel $60F_{254}$ of 0.2 mm thickness Merck) was used. The extract was applied as bands of 8 mm width and 6 mm distance in between tracks on a 6 x10 cm TLC plate. TLC scanner 030618 CAMAG) attached with WINCATS software were used for fingerprint development under UV 254/366 nm and after derivatization at 540 nm. CAMAG visualizer was used for photo documentation at UV 254 nm, 366 nm and invisible light after derivatization with vanillin-sulphuric acid reagent.

Procedure

The volumes of chloroform extract applied to the TLC plate were 5 µl, 10 µl, 15 µl. The extract was applied as 8 mm bands with 6 mm distance in between tracks and developed in the selected solvent system. The developed TLC plate was air dried and photographs were taken under UV 254 and 366 nm. The plate was scanned under UV 254 nm using the scanner. The fingerprint was recorded. Then the plate was dipped in vanillin-sulphuric acid reagent, heated in an oven at 105°C till the appearance of coloured spots. Immediately the photograph was taken and scanned for a fingerprint profile at 540 nm.

RESULTS AND DISCUSSION:

Macroscopic characters

Seeds are brown, elliptical-oblong, flat and fairly thick. They are sub orbicular and rugose. It measures 6 by 5mm.There is a prominent median ridge running from the base to the top **(Figure 2).**

FIGURE 2: SEEDS EXTERNAL FEATURES SHOWING MEDIAN RIDGE AND RUGOSE SURFACE (1X, 5X)

Microscopic characters

The seed consists of a thin testa seed-coat) and pair of thick plano convex cotyledons. The testa comprises outer sclerotesta sclerotic seed coat) and inner sarcotesta parenchymatous part). The sclerotesta is highly undulate with regular ridges and furrows throughout the seed surface **(Figure 3).**

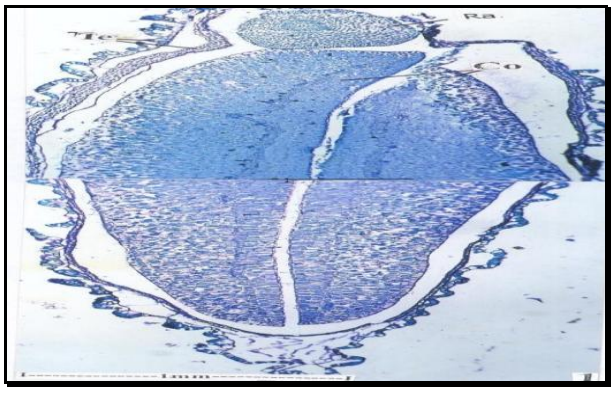


FIGURE 3: VERTICAL TANGENTIAL LONGITUDINAL SECTION OF THE SEED RECONSTRUCTED - 4X (CO: Cotyledons, RA: Radicle, TE: Testa**)**

The sarcotesta is thin and includes outer large, thin walled cell layer of parenchyma cells and inner thin layers of thick walled cells. The parenchymatous zone is thin along the lateral part of the seed and it becomes wider and many layered at the chalazal end **(Figure 4, 5).**

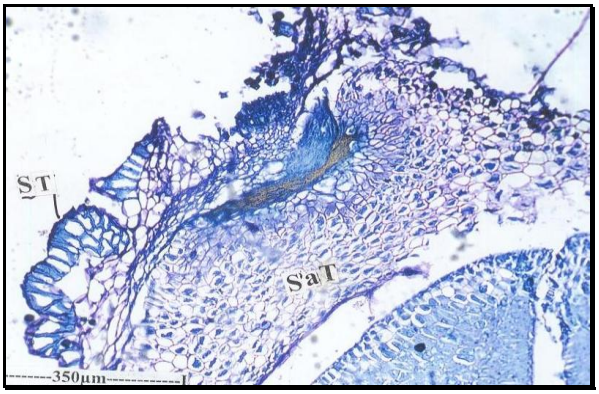


FIGURE 4: LS OF THE SEED UPPER CHALAZAL POSITION-10X

(SAT: Sarcotesta, ST: Sclerotesta)

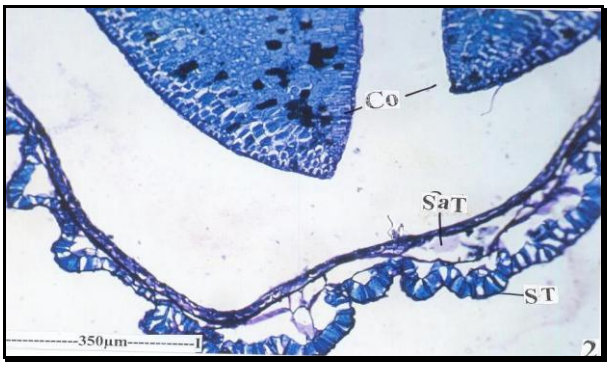


FIGURE 5: LOWER PORTION OF THE SEED SHOWS A PART OF THE COTYLEDONS AND SEED COAT-10X
(CO: Cotyledons)

Cells of the sarcotesta have dark cell inclusions. The sclerotesta which is much folded into ridges and wide furrows has this outer and inner tangential wall and spindle shaped much thickened radial walls **(Figure 6, 7).**

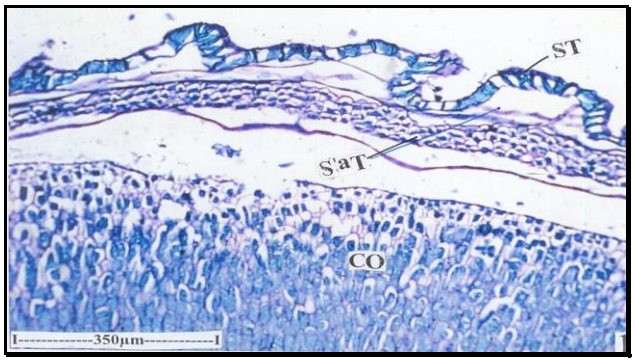


FIGURE 6: SECTIONAL VIEW OF THE SEED COAT-10X
(CO: Cotyledon, ST: Sclerotesta)

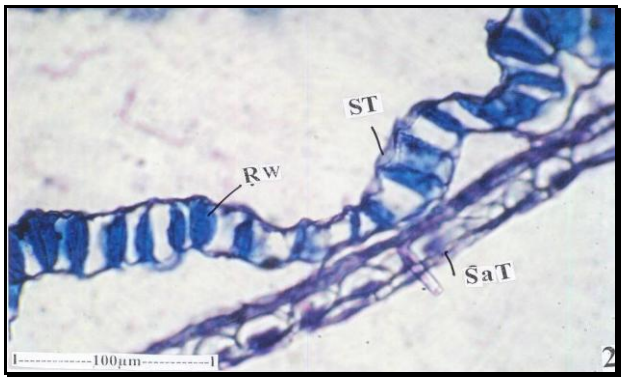


FIGURE 7: A PORTION OF THE COTYLEDONS-40X
(AT: Sarcotesta; RW: Radial wall-thick and lignified)

The thick radial walls are lignified. The sclero testa is 40 mm thick; the entire testa is 150 µm thick. The radicle is circular in sectional view which is 550 µm in diameter. It consists of young meristematic tissue and procambial strand. The cotyledons are plano convex, the flat sides facing each other. The cells of the cotyledons are parenchymatous and the cells include dense starch grains **(Figure 6, 3).**

Powder Microscopy

The fragments of seed coat are seen in surface view. The outer epidermis is the Sclerotesta which appears ameboid in outline due to thick, liquefied wavy anticlinal walls of sclerotesta. The cell lumen of the cells is wide and variable in outline **(Figure 8, 9).**

FIGURE 8: SEED COAT EPIDERMIS SCLEROTESTA) IN SURFACE VIEW-16X

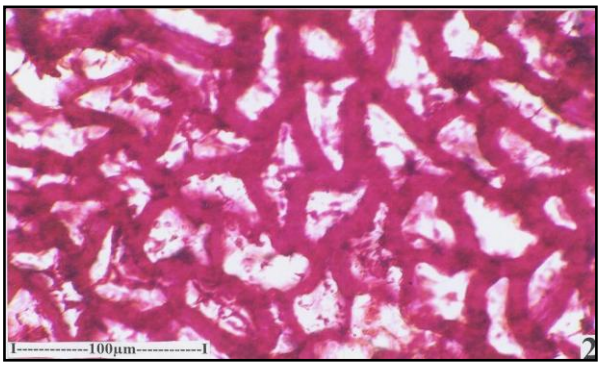


FIGURE 9: EPIDERMAL CELLS-ENLARGED-40X

Cells of sarcotesta are parenchymatous inner zone. The sarcotesta is parenchymatous. The cells are seen in small fragmentation. They are polyhedral in outline; the cell walls are thick and pit. The cells have dense amorphous dark stained inclusions **(Figure 10).**

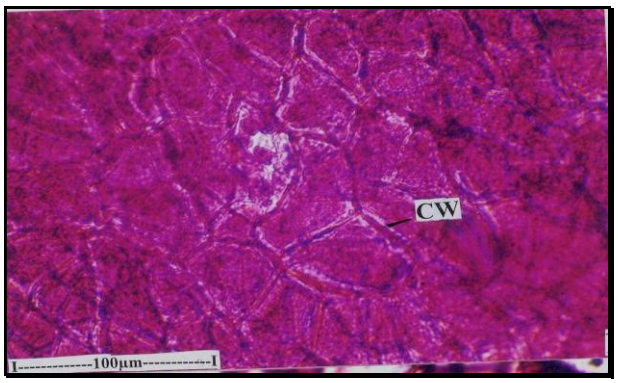


FIGURE 10: INNER PART OF THE SEEDS COAT SARCO-TESTA IN SURFACE VIEW-40X. CW: Cell wall

Broken pieces of the cotyledons are common in the powder. The cells are either squarish or rectangular. The cell walls are thick and straight. Starch grains are abundant in the cells. The cells are 20-30 x 10-15/nm in size **(Figure 11).**

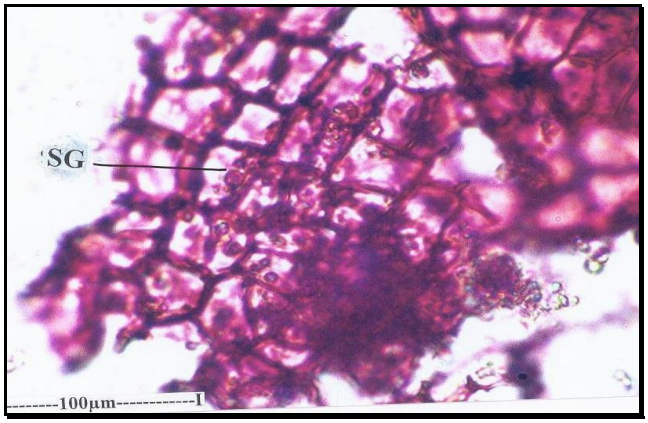


FIGURE 11: CELLS OF COTYLEDONS WITH STARCH GRAINS-40X
(SG: Starch grain)

Cotyledonary cells are also seen in a solitary condition. They are rectangular to squarish or triangular in outline. The cells are darkly stained (Figure 12).Starch grains are abundant in the cells. The cells are 20-30 x 10-15/nm in size **(Figure 11)**.

Cotyledonary cells are also seen in a solitary condition. They are rectangular to squarish or triangular in outline. The cells are darkly stained **(Figure 12).**

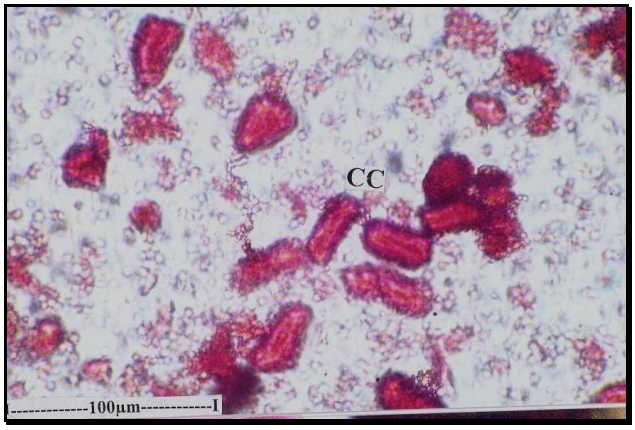


FIGURE 12: ISOLATED COTYLEDON-40X

Spherical shinning oil bodies of various sizes are seen in abundance in the powder. They do not stain with safranin. The bodies are small to large and are seen free floating in the water medium **(Figure 13)**.

Large, spherical starch grains are sparsely seen. They stain dark with IKI. The grains are mostly concentric with central hilum. They are up to 70 µm in diameter **(Figure 14).**

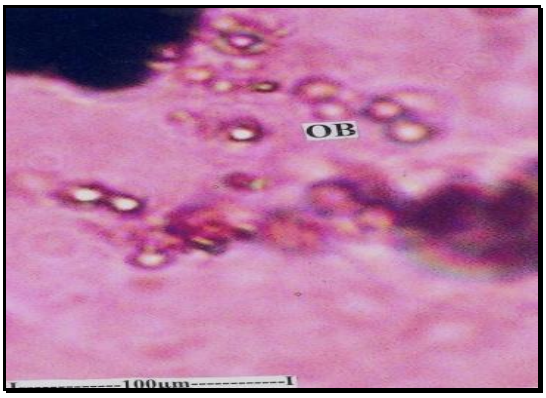


FIGURE 13: OIL-BODIES-40X

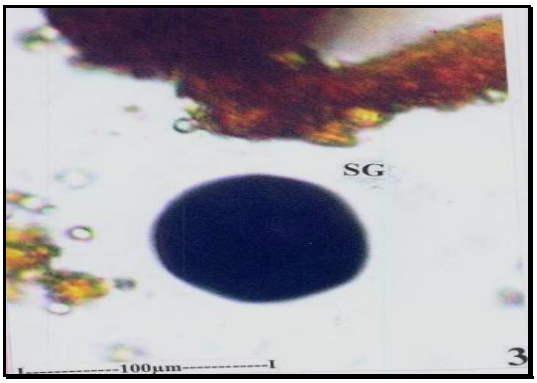


FIGURE 14. STARCH GRAIN-40X

Physico-chemical parameters

The loss on drying at 105^0C was calculated to be 6.80 % which may be due to the fixed oil present in the seed and the moisture may be negligible. The total ash was found to be 4.07 % which is indicating the presence of inorganic content in lower quantity. The acid insoluble ash was found to be 0.15 % which is very low. The water soluble extractive value and alcohol soluble extractive value were estimated to be 27.73 % and 19.45 % which indicates the presence of high polar compounds **(Table 1).**

TABLE 1: PHYSICO-CHEMICAL RESULTS OF *A. VASICA* SEED

Parameter %, w/w)	I	II	Mean
Loss on Drying at 105°C	6.90	6.70	6.80
Total Ash	4.05	4.09	4.07
Water soluble Ash	1.75	1.80	1.78
Acid insoluble Ash	0.15	0.15	0.15
Water Soluble Extractive	27.40	28.05	27.73
Alcohol Soluble Extractive	19.8	19.10	19.45

Preliminary phytochemical evaluation

The preliminary phytochemical test results revealed the presence of alkaloid, triterpene, flavonoid, phenol, saponin, steroid, coumarin, tannin and glycosides and absence of anthraquinone **(Table 2).**

TABLE 2: RESULTS PRELIMINARY PHYTOCHEMICAL OF *A. VASICA* SEED

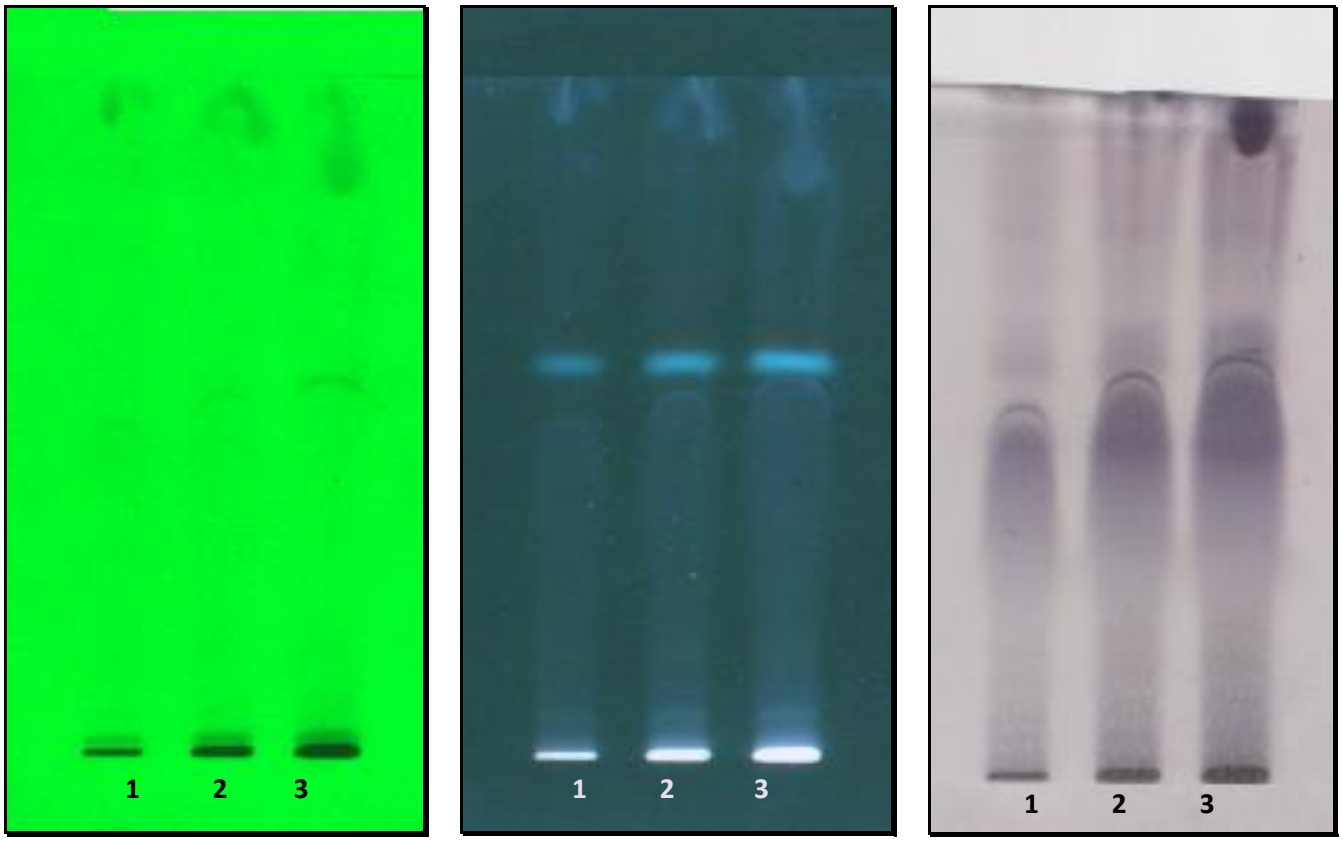
Qualitative Phytochemical Tests	Results
Alkaloids	+ ve
Triterpenes	+ ve
Flavonoids	+ ve
Phenols	+ ve
Saponin	+ ve
Steroids	+ ve
Anthraquinones	- ve
Coumarin	+ ve
Tannin	+ ve
Glycosides	+ ve

TLC/HPTLC study

The TLC photo documentation at UV 254 nm showed three spots at R_f values 0.03, 0.50 and 0.89; at UV 366 nm showed four spots at R_f values 0.06, 0.50, 0.58 and 0.89; and after derivatization with vanillin-sulphuric acid followed by heating showed four spots at R_f values 0.05, 0.53, 0.67 and 0.89. The spot in R_f value 0.89 is present in all conditions which may be movement of low polar compounds near to the solvent front **(Table 3, Figure 15A, 15B, 15C).**

TABLE 3: COLOUR AND R_F VALUES OF SPOTS UNDER PRE AND POST DERIVATIVE CONDITIONS

Under UV 254 nm		Under UV 366 nm		After Derivatization with Vanillin-Sulphuric acid	
R_f value	Colour of the spot	R_f value	Colour of the spot	R_f value	Colour of the spot
0.03	Green	0.06	Pale Blue	0.06	Violet
0.50	Green	0.50	Pale Blue	0.53	Violet
0.89	Green	0.58	Pale Blue	0.67	Violet
-	-	0.89	Pale Blue	0.89	Violet



A. Under UV 254 nm **B**.UV 366 nm **C.** After Derivatization

FIGURE 15: TLC PROFILE OF CHLOROFORM EXTRACT OF *A. VASICA* SEED

Track 1. 5µl; Track 2. 10 µl; Track 3. 15 µl.

The HPTLC finger print of *A. vasica* seed at UV 254 nm showed nine peaks at R_f values 0.19 11.85%), 0.63 44.54 %) and 0.81 21.91 %) which are major peaks and all other spots at R_f values 0.10, 0.24, 0.47, 0.70, 0.75 are minor peaks. 3D chromatogram at UV 254 nm of all three tracks

indicates the proportional increment in the peak heights. **(Table 4, Figure 16, 17)**.

TABLE 4: R_f AND % PEAK AREA OF CHLOROFORM EXTRACT OF *A. VASICA* SEED AT UV 254 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.09	0.6	0.10	16.0	5.08	0.12	0.3	183.0	3.57
2	0.16	0.6	0.19	22.5	7.18	0.22	8.5	607.3	11.85
3	0.22	8.7	0.24	13.0	4.14	0.27	3.1	308.5	6.02
4	0.46	4.0	0.47	29.0	9.24	0.48	6.6	189.0	3.69
5	0.60	8.9	0.63	168.0	53.47	0.66	13.6	2281.3	44.53
6	0.69	9.2	0.70	11.6	3.69	0.72	3.2	216.0	4.22
7	0.74	2.5	0.75	18.1	5.75	0.76	11.3	215.6	4.21
8	0.78	11.6	0.81	35.9	11.4	0.86	1.4	1122.8	21.91

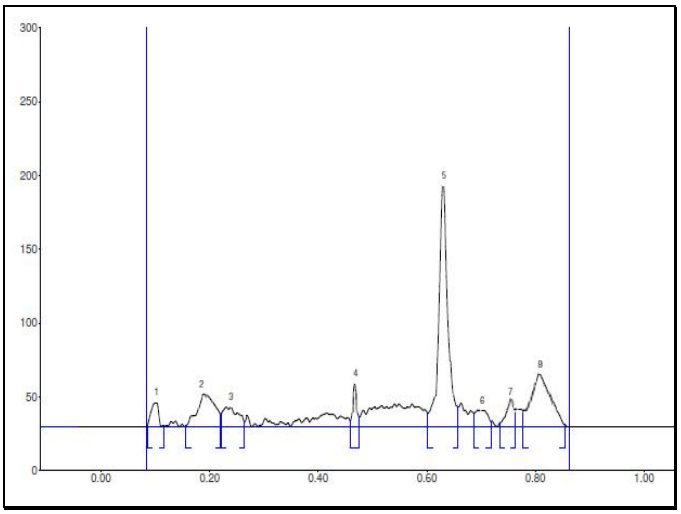


FIGURE 16: HPTLC FINGER PRINT PROFILE OF $CHCl_3$ EXTRACT OF *A.VASICA* SEED AT UV 254 nm

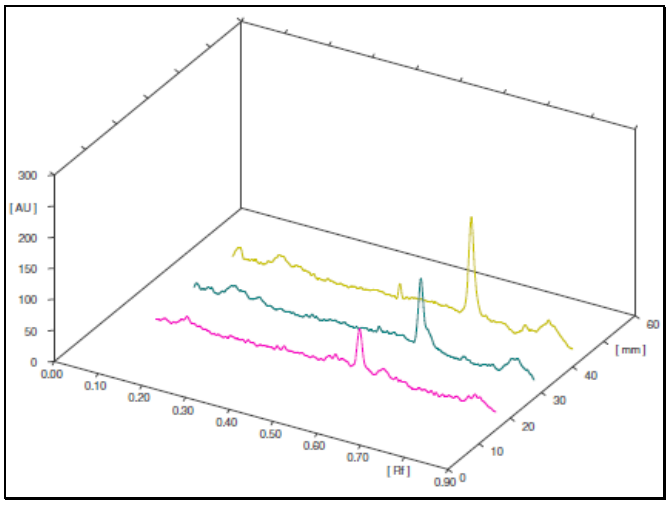


FIGURE 17: 3D OF CHROMATOGRAM OF $CHCl_3$ EXTRACT OF *A.VASICA* SEED AT UV 254 nm

The HPTLC finger print of *A. vasica* seed at UV 366 nm is showed only two peaks at R_f values 0.38(7.31 %) and 0.65 (92.69 %). 3D chromatogram at UV 366 nm of all three tracks are showed. **(Table 5, Figure 18, 19)**.

TABLE 5: R_f AND % PEAK AREA OF CHLOROFORM EXTRACT OF *A. VASICA* SEED AT UV 254 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.37	1.4	0.38	29.2.	25.37	0.39	1.5	155.4	7.31
2	0.60	1.5	0.65	85.8	74.63	0.69	2.5	1969.7	92.69

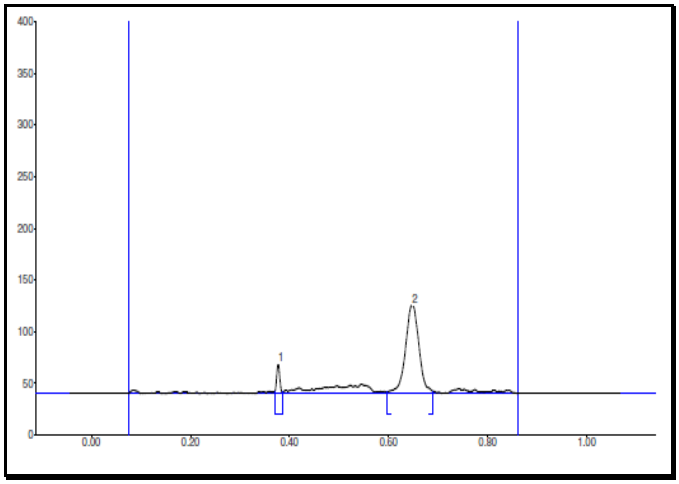


FIGURE 18: HPTLC FINGER PRINT PROFILE OF $CHCl_3$ EXTRACT OF *A. VASICA* SEED AT UV 366 nm

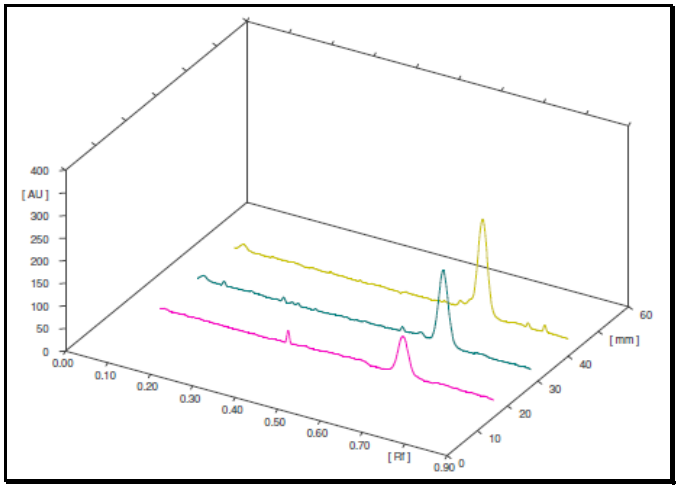


FIGURE 19: 3D CHROMATOGRAM OF $CHCl_3$ EXTRACT OF *A. VASICA* SEED AT UV 366 nm

The HPTLC finger print of *A. vasica* seed at 540 nm after derivatization with vanillin-sulphuric acid showed seven peaks at R_f values 0.07, 0.09, 0.15, 0.49 (80.79 %), 0.55, 0.61 (10.28 %) and 0.81 (1.21 %).3D chromatogram at 540 nm of all three tracks are showed. **(Table 6, Figure 20, 21)**.

TABLE 6: R_f AND % PEAK AREA OF CHLOROFORM EXTRACT OF *A. VASICA* SEED AT UV 540 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.06	0.1	0.07	15.8	2.83	0.08	1.7	151. 1	0.42
2	0.09	2.0	0.09	12.3	2.19	0.11	0.5	111.6	0.31
3	0.14	1.0	0.15	11.4	2.05	0.16	5.3	141.6	0.40
4	0.24	5.6	0.49	258.5	46.30	0.54	118.7	28751.6	80.79
5	0.54	118.9	0.55	153.8	27.54	0.57	32.8	2342.1	6.58
6	0.57	33.0	0.61	87.8	15.73	0.66	38.6	3658.5	10.28
7	0.78	1.5	0.81	18.7	3.35	0.84	1.2	431.5	1.21

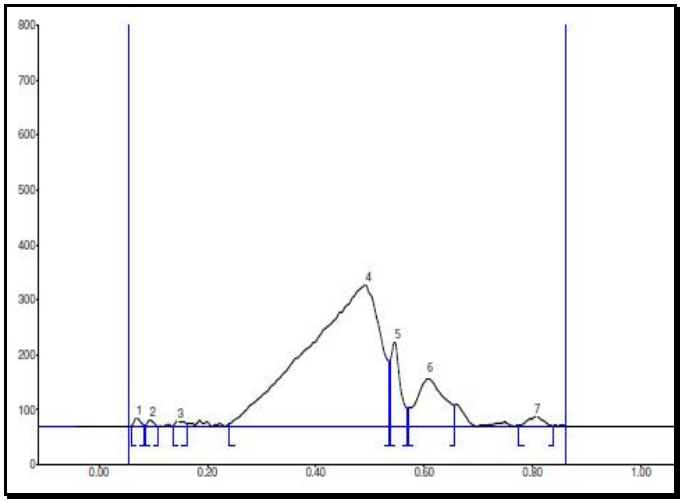


FIGURE 20: HPTLC FINGER PRINT PROFILE OF $CHCl_3$ EXTRACT OF *A.VASICA* SEED AT 540 nm

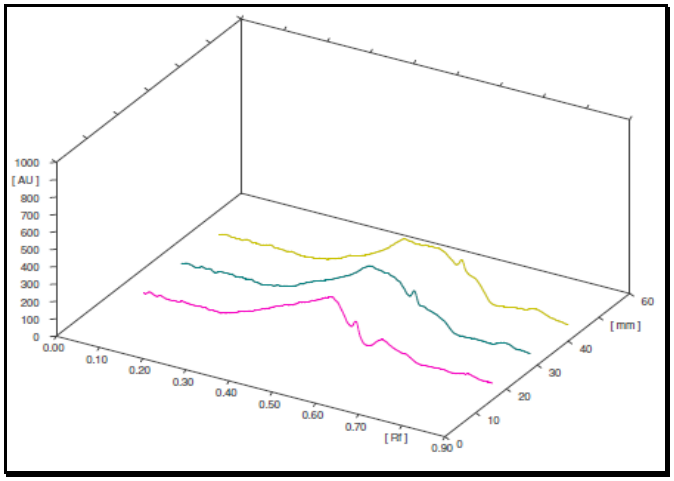


FIGURE 21: 3D CHROMATOGRAM OF ALL TRACKS OF $CHCl_3$ EXTRACT OF *A.VASICA* SEED AT 540 nm

CONCLUSIONS: The seed of *A. vasica* Nees. is being used as the raw material for the siddha medicines. The standards derived from the pharmacognostical, physic - chemical, phytochemical and TLC/HPTLC studies of the seeds of *Adhatoda vasica* Nees will be beneficial in framing the monograph of the drug in the Indian Pharmacopoeia.

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REFERENCES:

1. Shabir Lone A, Yadav AS, Ajit Sharma K, Malik Tafazul, Yogesh Badkhane and Raghuwanshi DK: A review on *Adhatoda vasica Nees*- An important and high demanded medicinal plant. Indo American Journal of Pharmaceutical Research 2013; 3:3.
2. Thokchom Singh P, Okram Singh M and Huidrom Singh B: *Adhatoda vasica Nees*: Phytochemical and Pharmacological Profile. The Natural Products Journal 2011; 1: 29-39.
3. Sunita Maurya and Dhananjay Singh: In vitro Callus Culture of *Adhatoda Vasica*: A Medicinal Plant. Annals of Biological Research 2010; 1:4:57-60.
4. Yamini K, Anto Shering M, Praveen Kumar Reddy S and Lakshmi Narasimha Reddy N: Pharmacognostical and Preliminary Phytochemical Screening on Leaves of *Trianthema decandra Linn.* International Journal of Pharmaceutical & Biological Archives 2011; 23:960-962.
5. Sass JE: Elements of botanical microtechnique. McGraw Hill Book and Co, New York, First Edition 1940: 222.
6. Johansen DA: Plant microtechnique. McGraw Hill Book and Co, New York, First Edition 1940: 523.
7. O'Brien TP, Feder N, Mc Cull ME: Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma* 1964; 59: 368-73.
8. Esau K: Anatomy of seed plants. John Wiley and Sons, New York, Fourth Edition 1979: 550.
9. Anonymous, Quality Control Methods for Medicinal Plant Materials. World Health Organization, Geneva, 1998.
10. Harbone JB: Phytochemical Methods. Chapman and Hill, London, 1973.
11. Trease GE, Evans WC: Pharmacognosy. Brailliar Tiridel and Macmillian Publishers, London, Eleventh Edition 1989.

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