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PHARMACOGNOSTIC STANDARDIZATION AND HPTLC FINGERPRINT PROFILE OF *TRICHOSANTHES DIOICA* Roxb. FRUITS

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ABSTRACT

Trichosanthes dioica Roxb. is commonly known as Parwal, fruits of which are used as vegetable all over India and different parts of this plant are used in the traditional medicine to treat various types of human ailments. The current study was therefore carried out to provide requisite pharmacognostic details about the fruit of *T. dioica* Roxb. Pharmacognostic evaluation included examination of macroscopical and microscopical characters, physicochemical properties, phytochemical analysis, fluorescence study and HPTLC fingerprint profile. The powder microscopy showed the presence of sclerenchymatous cells, xylem vessels with spiral thickening, fibers, starch grains, stone cells and trichomes. Phytochemical screening showed the presence of glycosides, steroidal compounds, flavonoids and saponins. Qualitative densitometric HPTLC fingerprint profile of methanolic extract can provide standard finger prints and can be used as a reference for the identification and quality control of the fruit. The present study will provide the information with respect to identification and authentication of *Trichosanthes dioica* Roxb. fruits.

KEYWORDS: *Trichosanthes dioica* Roxb., fruits, pharmacognosy, phytochemical analysis, HPTLC fingerprint profile.

INTRODUCTION

Plants have formed the basis for the treatment of diseases in traditional medicine systems since ancient time and continue to play a major role in the primary health care. Moreover, natural products and plant-derived products continue to be excellent source of new drugs. Over the centuries, the use of medicinal herbs has become an important part of daily life

despite the progress in modern medical and pharmaceuticals research. Traditional medicine is based on various systems including Ayurveda, Siddha and Unani use herbs for treatment.[1]

According to WHO more than one million people rely on herbal medicines to some extent. India has an ancient heritage of traditional medicine. Approximately 3000 plant species are known to have medicinal properties in India.[2] The Rigveda (3700 B.C) mentions the use of medicinal plants. Materia Medica of India provides lots of information on the folklore practices and traditional aspects of therapeutically important natural products.[3]

Herbal drugs have great growth potential in the global market. Natural product research continues to explore Indian traditional medicines to develop novel drugs. There is a great demand for herbal medicines in the developed as well as developing countries because of their wide biological activities, higher safety margin than the synthetic drugs and lower costs.[4] Therefore it is essential to ensure reproducible quality of herbal products. The evaluation of these drugs is mostly based on phytochemical, pharmacological and allied approaches including various instrumental techniques like microscopy, chromatography and others.[5]

Trichosanthes dioica Roxb. (family: Cucurbitaceae), commonly known as "Sespadula" in English and "Parwal" in Hindi, is widely grown throughout India.[6] Fruits of this plant are used as vegetable in Indian traditional food system from time immemorial. Besides fruits, other parts of the plant, such as the leaves and tender shoots, have also been used in the traditional system of medicine since ancient times.[7,8,9] Over 20 species have been recorded in India of which two namely *T. anguina and T. dioica* are cultivated as vegetable. Other important species found in the world are *T. palmata, T. cordata, T. nervifolia, T. cucumerina, T. wallichiana, T. cuspida, T.incisa, T. laciniosa* and *T. kirilowii*etc.[10]

Trichosanthes dioica Roxb. has been mentioned in various Ayurvedic texts in the treatment of such life style diseases. The plant has a promising place in Ayurvedic system of medicine due to its various medicinal values like antidiabetic, anthelmintic, antiglycemic, anti inflammatory properties. The plant is rich in Vitamin A, Vitamin C, Tannins, Saponins, alkalodis and tetra and pentacycline triterpenes.[11] In spite of the numerous medicinal uses attributed to fruit, there is no detailed pharmacognostic report is available from literature; therefore, detailed pharmacognostic studies comprising macroscopy, microscopy, powder characteristics, physicochemical analysis and preliminary phytochemical analysis of

Trichosanthes dioica Roxb. fruit was undertaken to evaluate and establish quality control standards as per Pharmacopoeia of India and WHO guidelines, which will help in identification as well as in standardization.[12,13]

The WHO accepts HPTLC fingerprint profile as an identification and quality evaluation technique for medicinal herbs since 1991.[14] HPTLC fingerprint can be a unique identification utility for herbs and their different species.[15,16] Therefore, HPTLC fingerprint profile for *Trichosanthes dioica* Roxb. fruit has been also developed.

MATERIALS AND METHODS

Herbarium of *Trichosanthes dioica* Roxb. was prepared and authenticated from Blatter Herbarium, St. Xavier's College, Mumbai. Fresh fruits of *T. dioica* Roxb. were collected from Badlapur and Karjat, M.S., India, washed under running tap water and blotted dry. The fruits were then cut into small pieces and kept for drying in pre-set oven at 40± 2º C for five days and ground into powder and used for further analysis.

Physicochemical constants such as the percentage of total ash, acid insoluble ash, water soluble ash; water soluble and alcohol soluble extractive values were calculated according to the methods described in Indian Pharmacopoeia.[17] Fluorescence analysis was conducted using methods of Kokoski (1958)[18] and Chase and Pratt (1949).[19]

Preliminary phytochemical analysis of fruit extracts was performed as described by Khandelwal (1998)[20] and Kokate (2007).[21] Phytochemical analysis was also carried out using Thin Layer Chromatography as per methods described by Wagner and Bladt (1996).[22]

A qualitative densitometric HPTLC analysis was performed for methanolic fruit extract to develop characteristic fingerprint profile, which may be used for quality evaluation. 10 µl of extract was spotted on pre-coated silica gel 60 F_{254} HPTLC plates (Merck) with the help of CAMAG Linomat V applicator. The plate was developed in glass twin trough chamber (20 cm x 10 cm) pre-saturated with mobile phase (Toluene: Ethyl acetate: Methanol: Glacial Acetic acid in the ratio 7.5:1.5:0.75:0.2). The plate was derivatized using methanolic H_2SO_4 and scanned using TLC Scanner 3 (CAMAG).

RESULTS

Macroscopy

Fruit: *T. dioica* Roxb. fruit is a berry, which is globose, oblong or ovoid to ellipsoid or fusiform, smooth and green in colour, 7.0-9.0 cm (Avg. 7.0 cm ± 0.38) long and 1.8-2.2 cm (Avg. 2 cm ± 0.41) in diameter. The fruit has a thick rind and fleshy pulp (Plate no.1A).

Seed

Many seeds are embedded in fleshy pulp. The seeds are ovate or rounded, 1-loculed, compressed, turgid. The testa is smooth, yellowish to white, black or dark brown, without distinct margin (Plate no.1B).

Microscopy

Surface preparation of the fruit shows presence of anamocytic type of stomata. They have rather large guard cells and thin walled accompanying cells (Plate no. 1C).

Transverse section of fruit

T.S. of fruit shows presence of exocarp and mesocarp. Epidermis shows presence of trichomes. Mesocarp shows outer layer containing stone cells and central parenchymatous region with seeds embedded in it, parenchymatous region of mesocarp shows presence vascular bundles, which are bi-collateral. Starch grains (spherical) are also observed especially in inner part of mesocarp. The parenchymatous region of the mesocarp near the layer of stone cells shows presence of some cells containing yellow colored pigments (Plate no. 1D - G).

Transverse section of seed

T.S. of the seed shows testa. The outer part of testa is composed of few rows of thick walled lignified cells forming a compact mass, inner part of testa consist of thick walled parenchyma. Inner to testa is endosperm showing presence of cells containing starch grains encircling embryo axis (Plate no. 1H and 1I).

Powder Microscopy

The study of fruit powder shows presence of sclerenchymatous cells, xylem vessels with spiral thickening, fibers, starch grains, stone cells and trichomes (Plate no. 1 J-P).

Physico-chemical analysis

Ash of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drugs results in an ash residue consisting of an inorganic material (metallic salts and silica). This value varies within fairly wide limits and is therefore an important parameter for the purpose of evaluation of crude drugs.[23] Therefore, percentage of the total ash, acid insoluble ash and water soluble ash were determined. The extraction of any crude drug with a particular solvent yields an extract containing different phytoconstituents. Extractive value is also useful for evaluation of crude drug, which gives an idea about the nature of the chemical constituents present in a crude drug and is useful for the estimation of specific constituents, soluble in that particular solvent used for extraction.[20] Results are tabulated in Table 1.

Fluorescence analysis

The fluorescence characters of powdered drug plays a vital role in the determination of quality and purity of the drug material. The powdered drugs exhibit characteristic fluorescence in the presence of different chemical reagents under ultraviolet light.[24] The change in the colour of the fruit powder under UV radiation (254 and 366 nm) with reference to day light was observed. Results of fluorescence analysis are tabulated in Table 2.

Phytochemical analysis

The preliminary phytochemical screening of fruit powder was carried out using various solvents *viz.* water, ethanol, methanol, benzene and petroleum ether. These extracts were subjected to various qualitative chemical analysis showed presence of aleurone grains, amino acids, proteins, carbohydrates, starch fats and fixed oils; alkaloids, glycosides, mucilage, tannins, steroids, phenols, flavonoids, saponins, essential oils and resins whereas acid compounds and anthraquinone were not detected. The results are depicted in Table 3.

Phytochemical studies using Thin Layer Chromatography revealed the presence of anthracene derivatives, arbutin derivatives, bitter principles, cardiac glycosides, coumarin derivatives, essential oils, lignans, pungent-tasting principles, saponins, triterpenes and valepotraites class of compounds. (Table 4; Plate no. 2).

HPTLC fingerprint profile

HPTLC, now a days is applied to obtain “Fingerprint” patterns of herbal formulations, quantification of active ingredients and also detection of adulteration.[25] HPTLC fingerprint

profile of methanolic fruit extract showed distinct band pattern before and after spraying with derivatizing reagent methanolic sulphuric acid. *Rf* values under different wavelengths before and after derivatization are tabulated in Table 5 (Plate no. 3).

DISCUSSION

To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.[24] The standardization of crude drug is an integral part of establishing its correct identity for inclusion of crude drug in Pharmacopoeia. Thus, in recent years, there has been an emphasis on standardization of medicinal plants of therapeutic potential. Despite the modern techniques, identification and evaluation of plant drugs by pharmacognostical study is still more reliable, accurate and inexpensive means.[26] The results obtained from the present investigation could, therefore, serve as a basis for proper identification, collection and investigation of the plant.[27]

The present study is focused on pharmacognostic features of *T. dioica* Roxb. fruits and physicochemical parameters of the fruit powder. The organoleptic and macroscopic studies obtained important characteristic features of the fruit. The physicochemical standards, such as ash values, extractive values will be useful to identify the authenticity of the drug even from the powdered fruit sample. It will also serve as a standard data for the quality control of the herbal preparations containing this fruit. Using these standards, the plant can be differentiated from other related species.[28]

The fluorescence method is adequately sensitive and enables the precise and accurate determination of the analytes over a satisfactory concentration range without several time consuming dilution steps prior to analysis of pharmaceutical samples.[29] Kalidas *et al.* (2009) suggested that a non-fluorescent compound may fluoresce if mixed with impurities that are fluorescent.[30] Therefore, the results obtained from the present fluorescence studies will also help to check any impurities present in fruit powder of *T. dioica* Roxb.

Preliminary phytochemical and TLC analysis showed presence of different phytochemical compounds such as carbohydrates, proteins, amino acids, tannins, hydrolysable tannins, bitter principles, essential oils, valepotraites, coumarins, flavonoids and terpenes, which could make the fruits useful for treating different ailments. Thus the preliminary screening tests

may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development.[28]

HPTLC is one of the simplest and modern techniques available today, which provides a chromatographic fingerprint and is suitable for confirming the identity and purity of plants and for detecting adulteration and substitution. HPTLC fingerprint profile along with their recorded *Rf* values, can serve as reference standard for further research on the medicinal properties of the fruit.[27]

CONCLUSION

Majority of the people, even today still rely on their traditional materia medica (medicinal plants and other materials) for their everyday health care needs. In recent years there has been an emphasis on standardization of medicinal plants of therapeutic potential. Despite the modern techniques, identification and evaluation of plant drugs by pharmacognostic studies is still more reliable, accurate and inexpensive means. Since *T. dioica* Roxb. fruits are known for its various medicinal properties, the present study could be useful to supplement information with respect to its identification, authentication and standardization. The information generated can also be useful for preparation of monograph of the plant, which could be incorporated in the preparation of Indian Herbal Pharmacopoeia.

Table 1: Physico-chemical analysis of *T. dioica* Roxb. Fruits.

Sr. No.	Parameters	% ± SD
1.	Loss on drying	89.70 ± 0.29
2	Ash Values	
	a. Total Ash	5.71± 0.05
	b. Acid insoluble	0.8 ± 0.15
	c. Water soluble	4.31 ± 0.10
3	Extractive values	
	Water soluble	29.33 ±0.45
	Alcohol soluble	16 ± 0.57

Table 2: Fluoresence analysis of *T. dioica* Roxb. fruit powder.

Sr. No.	Test	Ordinary Light	U.V. Light	
		540 nm	254 nm	366 nm
1.	Powder as such	Brown	Buff Brown	Fluroscent white
2.	Powder + Nitrocellulose	Light brown	Gray	Light green
3.	Powder + 1N NaOH in methanol	Yellowish brown	Black	Yellowish green
4.	Powder + 1N NaOH in methanol+ nitrocellulose in amylacetate	Chrome Yellow	Black	Yellowish green
5.	Powder + 1 N HCl	Light brown	Buff brown	Light green

6.	Powder + 1N HCl + nitrocellulose in amylacetate	Dark yellow	Gray	Light green
7.	Powder + 1N NaOH in water	Chrome yellow	Green	Light green
8.	Powder + 1N NaOH in water+ nitrocellulose in amylacetate	Chrome yellow	Black	Buff brown
9.	Powder + HNO_3 (1:1)	Dark brown	Brown	Green
10.	Powder + H_2SO_4 (1:1)	Brownish green	Dark green	Light green
11.	Powder + 1% Picric acid	Yellow	Brown	Green
12.	Powder + Acetic acid	Yellowish brown	Gray	Gray
13.	Powder + 5% Iodine	Yellow	Gray	Light green
14.	Powder + 5 % $FeCl_3$	Brown	Black	Black
15.	Powder + 25 % NH_3 + HNO_3	Brown	Gray	Light green
16.	Powder + Methanol	Brown	Gray	Gray
17.	Powder +Conc. HNO_3	Brown	Gray	Light green
18.	Powder + 10% $K_2Cr_2O_7$ solution	Orange	Black	Brown
19.	Powder + 50% KOH	Yellow	Black	Light green

Table 3: Preliminary Phytochemical analysis of various extracts of *T. dioica* Roxb. Fruit.

Sr. No.	Phytoconstituent	AE	EE	ME	BE	PEE
1.	Acid compounds	ND	ND	ND	ND	ND
2.	Aleurone grains	+	ND	ND	ND	ND
3.	Alkaloids	ND	ND	ND	+	+
4.	Amino acid	+	+	+	ND	ND
5.	Proteins	+	+	+	ND	ND
6.	Carbohydrates	+	+	+	ND	ND
7.	Starch	+	+	+	ND	ND
8.	Fats & fix oils	ND	+	+	+	+
9.	Glycosides	+	+	+	+	+
10.	Mucilage	+	+	+	ND	ND
11.	Tannins	+	+	+	ND	ND
12.	Steroids	ND	+	+	+	+
13.	Phenols	+	+	+	ND	ND
14.	Flavonoids	+	+	+	ND	ND
15.	Saponins	+	+	+	ND	ND
16.	Essential Oils	ND	+	+	ND	+
17.	Resins	+	ND	ND	ND	ND
18.	Anthraquinone	ND	ND	ND	ND	ND

KEYWORDS: +: present; ND: Not Detected; AE: Aqueous extract; EE: Ethanolic extract; ME: Methanolic extract; BE: Benzene extract; PE: Petroleum ether extract.

Table 4: Phytochemical analysis of *T. dioica* Roxb. fruit by TLC.

Sr. No.	Phytoconstituents	Derivatizing Agent	Band colour observed
1.	Anthtracene derivatives	Potassium Hydroxide reagent	Red Colour Fluorescence (366nm)
2.	Arbutin derivatives	Gibb's reagent	Blue-Violet Colour (540 nm)
3.	Bitter principles	Anisaldehyde – Sulphuric acid reagent	Red – Violet, Brown – Red, Blue – Green And Blue Colour (540 nm)
4.	Cardiac glycosides	Sulphuric acid reagent	Blue and Brown colour (540 nm)
5.	Coumarin derivatives	Potassium hydroxide reagent	Blue Colour Fluorescence (366nm)
6.	Essential Oils	Anisaldehyde-Sulphuric acid reagent	Blue colour (540 nm)
7.	Lignans	Sulphuric acid reagent	Red-violet colour (540 nm)
8.	Pungent Tasting principles	Vanillin – Sulphuric acid reagent	Blue - violet colour (540 nm)
9.	Saponins	Vanillin – Sulphuric acid reagent	Blue, Blue – Violet, Red and Yellow – Brown colour (540 nm)
10.	Triterpenes	Anisaldehyde-Sulphuric acid reagent	Blue – Violet And Red – Violet colour (540 nm)
11.	Valepotraites	Anisaldehyde-Sulphuric acid reagent	Violet and Blue colour (540 nm)

Table 5: HPTLC fingerprint profile (*Rf* values) of methanolic extract of *T. dioica* Roxb. Fruit.

Sr. No.	Before Derivatizing		After Derivatizing
	254 nm	366 nm	540 nm
1.	0.09	0.10	0.09
2.	0.14	0.19	0.13
3.	0.19	0.25	0.19
4.	0.25	0.29	0.21
5.	0.29	0.37	0.25
6.	0.37	0.47	0.30
7.	0.44	0.58	0.34
8.	0.55	0.76	0.45
9.	0.61	0.81	0.55
10.	0.68	-	0.69
11.	0.75	-	0.72
12.	0.84	-	0.82
13.	-	-	0.84

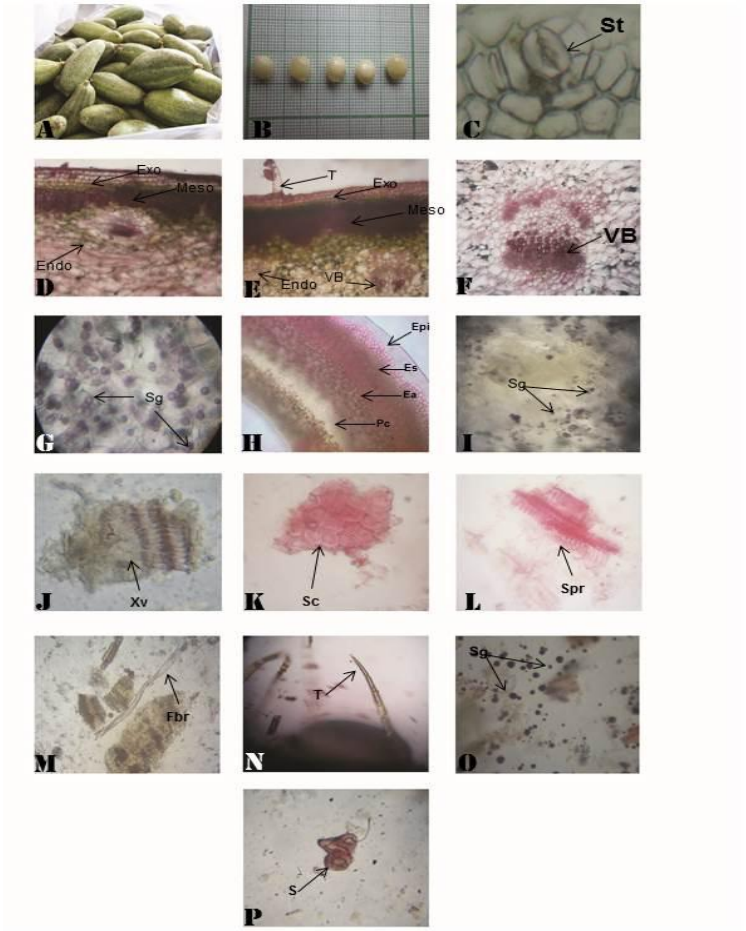

Plate no. 1: Macroscopic, microscopic and powder characteristic of *Trichosanthes dioica Roxb.* Fruits.

A: Fruits, B: Seeds, C: Epicarp in surface view showing stomata (St), D: T. S. of fruit showing Exo- Exocarp, Meso- Mesocarp, Endo- Endocarp, E: T. S. of fruit showing T- Trichomes, Exo- Exocarp, Meso- Mesocarp, Endo- Endocarp, VB- Vascular bundle, F: T. S. of fruit showing vascular bundle (VB), G: Parenchymatous cells showing starch grains (Sg), H: Transverse section of seed showing Epi- Epidermis, Es – Endosperm, Ea- Embryo axis, Pc- Parenchymatous cells, I: Transverse section of seed showing starch grains (Sg) in endosperm, J: Powder showing xylem vessels (Xv) traversing parenchyma, K: Powder showing sclerenchymatous cells (Sc), L: Powder showing spiral thickening (Spr) M: Powder showing fibre (Fbr), N: Powder showing trichomes (T), O: Powder showing starch grains (Sg), P: Stone cell (S)

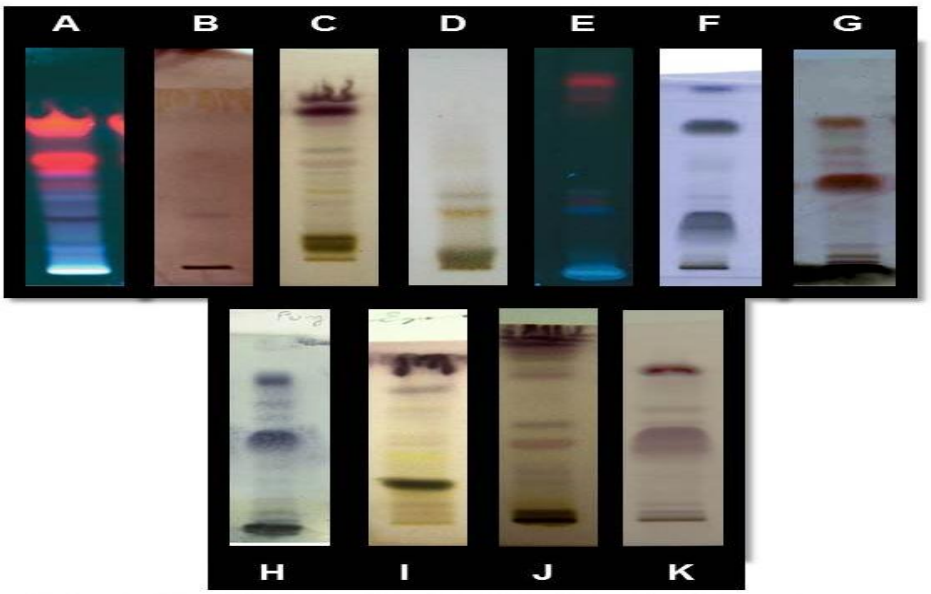

Plate no. 2: Phytochemical analysis using TLC of *Trichosanthes dioica Roxb.* Fruits.

A: Anthtracene derivatives, B; Arbutin derivatives, C: Bitter principles, D: Cardiac glycoside, E: Coumarin derivatives, F: Essential Oils, G: Lignans, H: Pungent Tasting Principles, I: Saponins, J: Triterpenes, K: Valepotraites

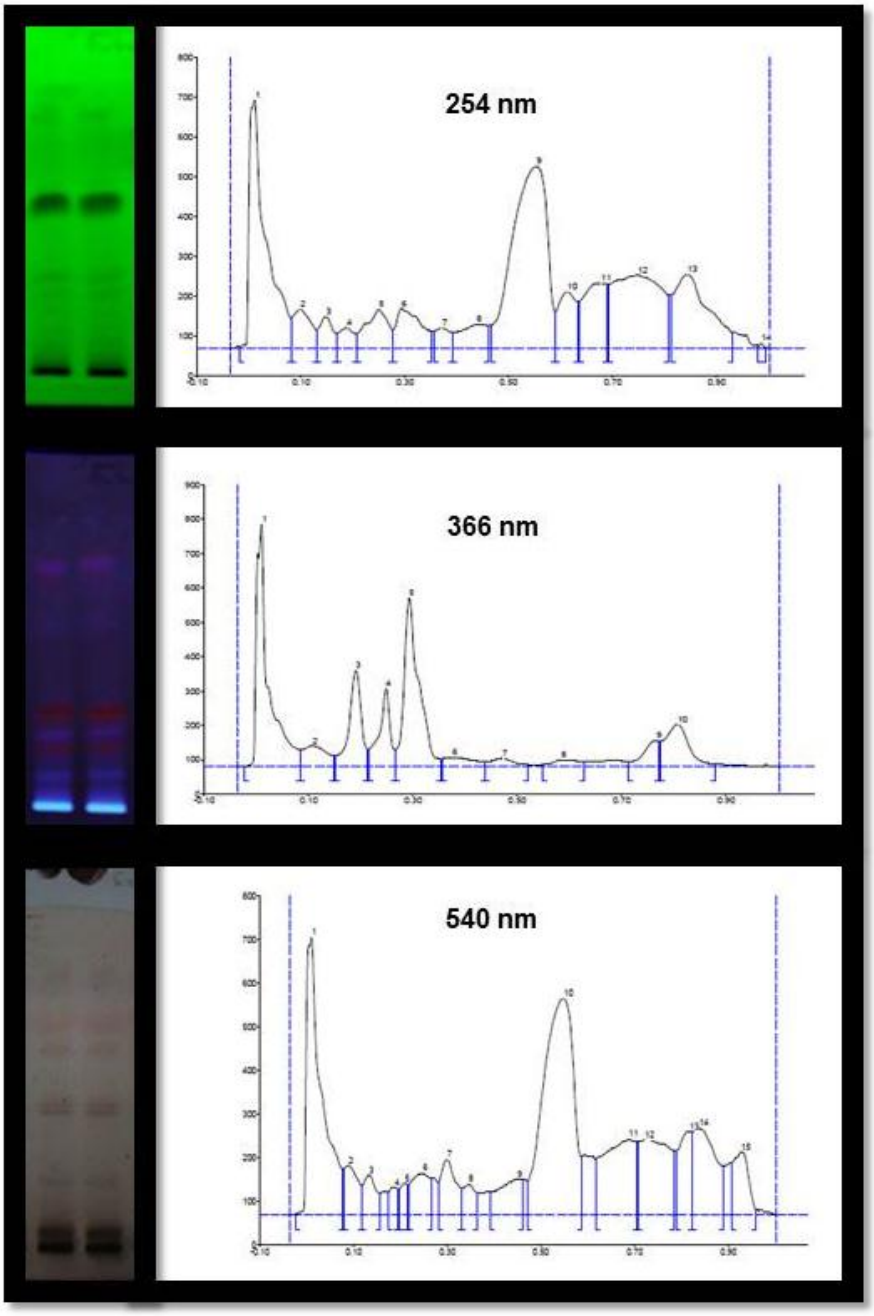

Plate No. 3: HPTLC Fingerprint of *T. Dioica* Roxb. Fruits

REFERENCES

1. World Health Organization. Guidelines for the Assessment of Herbal Medicine Programme on Traditional Medicine. WHO/TRM/91.4.WHO, Geneva, 2003.
2. Prakasha, H.M., Krishnappa, M., Krishnamurthy, Y.L., Poornima, S.V., Folk medicine of NR PuraTaluk in Chikamaglur district of Karnatka. Indian J Trad Knowl, 2010; 9(1): 55-60.

3. Kirtikar KR, Basu BD, Indian Medicinal Plants, Lalit Mohan Basu Publications, Allahabad, 1980.
4. Gadre AY, Uchi DA, Rege NN, Daha SA, Nuclear Variations in HPTLC Fingerprint Patterns of Marketed Oil Formulations of *Celastrus paniculates*., Ind. J. of Pharmacology, 2001; 33: 124-45.
5. Paarakh, P.M., *FicusRacemosa* Linn. – An overview., Natural Product Radiance, 2009; 8(1): 84-99.
6. Chakravarthy, H.M., Fascicles of flora of India - 11 Cucurbitaceae. Botanical Survey of India, 1982; 136.
7. Sharma, G., and M.C. Pant. Effects of feeding *Trichosanthes dioica* (parval) on blood glucose, serum triglyceride, phospholipid, cholesterol, and high density lipoprotein-cholesterol levels in the normal albino rabbit. Current Sci., 1988; 57: 1085–1087.
8. Sharma G, Sarkar A, Pachori SB, Pant MC. Biochemical evaluation of raw *Trichosanthes dioica* whole fruit and pulp in normal and mild- diabetic human volunteers in relation to lipid profile. Ind Drug, 1989; 27: 24–28.
9. Singh, K. Pointed gourd (*Trichosanthes dioica* Roxb.). Indian Hort., 1989; 33: 35–38.).
10. Kumar N. *Trichosanthes dioica* Roxb: an overview. Int. J. Pharm. Bio. Sci, 2011; 3: 111–118.
11. Gupta V, Pagoch SS, Phytopharmacological Review of *Trichosanthes Dioica* (Patola) IAMJ, 2014; 2(3): 322-327.
12. Pharmacopoeia of India: Ministry of Health and Family Welfare, Govt. of India. New Delhi. 1996; 2: A-53, 54, 89, 95.
13. WHO, Quality Control Methods for Medicinal Plant Materials, Geneva, 1998; 9: 22-24, 33.
14. WHO, Guidelines for the Assessment of Herbal Medicine. Munich, 1991.
15. Anonymous, CHMP. Guideline on Quality of Herbal Medicinal Products/ Traditional Herbal Medicinal Products. Committee for Medicinal Products for Human Use, European Medicines Agency Inspections, http://www.emea.eu.int; 2005.
16. Welsh WJ, Lin WK, Tersigni SH, Collantes E, Duta R, Carey MS, Pharmaceutical fingerprinting: evaluation of neural networks and chemometric techniques for distinguishing among same product manufacturers. Anal Chem., 1996; 68: 3473 -3482.
17. Indian Pharmacopoeia. Government of India. Ministry of Health and Family Welfare. Ghaziabad: Indian Pharmacopoeia Commission, 2007.

18. Kokoski J, Kokoski R, Salma FJ, Fluorescence of powdered vegetable drugs under ultraviolet radiation, J Am Pharm Assoc, 1958; 47: 715-717.
19. Chase CR, Pratt RJ, Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification, J Am Pharm Assoc, 1949; 38: 324-333.
20. Khandelwal KR, Practical Pharmacognosy: Techniques and Experiments. 4th ed. India: Nirali Prakashan, 1998.
21. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 37th ed. Nirali Prakashan, 2007.
22. Wagner H, Bladt S, Plant Drug Analysis: A Thin Layer Chromatography Atlas. Berlin: Springer; 1996.
23. Mukharjee PK, Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals. India: Business Horizones, 2005.
24. Kumar V, Yadav PS, Singh PU, Bhat HR, Rana A, ZamanMdK. Pharmacognostical evaluation of *Cuscuta reflexa* Roxb., Phcog J., 2010; 2: 74-82.
25. Sanja SD, Sheth NR, Patel NK, Patel D, Patel B, Characterization and evaluation of antioxidant activity of *Portulaca oleracea* L., Int J Pharm Pharm Sci., 2009; 1: 74-84.
26. Pandya H, Kachwala Y, Sawant L, Pandita N, Pharmacognostical screening of seeds of *Cassia absus*, Phcog J, 2010; 2(11): 419-426.
27. Patil AG, Koli SP, Patil DA, Naresh C, Pharmacognostical standardization and HPTLC fingerprint of *Crataeva tapia* Linn. SSP. Odora (Jacob.) Almeida Leaves, Int J Pharm Biol Sci., 2010; 1: 1-14.
28. Rabari H, Pandya S, Vidyasagar G, Gajra B, Pharmacognostical and phytochemical investigation of *Cocculus pendulus* (J.R. and G. FORST.) Diels Leaf, Int J Pharm Biol Sci., 2010; 1: 1-13.
29. Brindha P, Sasikala P, Purushothaman KK, Pharmacognostic studies on *Merugan Kizhangu* Bull. Med. Eth. Bot. Res., 1981; 3: 84-96.
30. Kalidass C, Mohan VR, Abragam AD, Pharmcognostic studies on *Capparis sepiaria* (L.) R.Br., Phcog J., 2009; 1: 121-125.