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Research Article

Pharmacognostic and Phytochemical Evaluation of *Trichosanthes dioica* (R.)

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ABSTRACT
Trichosanthes dioica seeds are mentioned in various traditional texts as a drug used for vermicidal anthelmintic, insecticidal, sedative, diuretic, demulcent, and expectorant purpose ethnopharmacologically. The studies were taken up to evaluate pharmacognostic, physicochemical & phytochemical standard for *Trichosanthes dioica* seeds. The objective of present study is to evaluate the morphological, microscopical, phytochemical and physicochemical properties of various bioactive compounds present in *Trichosanthes dioica* seeds. Preliminary phytochemical screening was done and HPTLC studies were carried out. CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner, and WIN CATS-4 software were used. The microscopical studies of *T. dioica* seeds have showed mucilaginous epidermis made up of long thin trichomes, Innermost layer of parenchyma cells and sclerotic endodermal layer. Testa is 17-23 cells thick on the sides on the seeds. Exotesta: a layer shortly columnar pulpy cells, much elongate on the sides of micropyle thin walled but with fine fibrillar thickenings (not lignified) on the radial and inner wall, the outer wall thickened and slightly lignified, first filled with starch grains. Physico-chemical studies of *T. dioica* seeds have set the some standard i.e. Ether soluble extractive value 16.15%w/w, alcohol soluble extractive value 10.11% w/w, water soluble extractive value 9.22% w/w, Total Ash value 6.21 w/w, acid insoluble ash value 1.32% w/w, water soluble ash value 4.29% & loss on drying 24.33% w/w etc. were found out. Preliminary phytochemical screening was done then TLC and HPTLC studies were carried out. All the findings will be useful towards establishing pharmacognostic standards on identification, purity, quality and classification of the plant, which is gaining relevance in plant drug research for the identification and preparation of monograph of plant.

keywords: Anthelmintic, Phytoconstituents, *Trichosanthes dioica*, Pharmacognostic.

INTRODUCTION

Trichosanthes dioica is an easily available common plant. Apart from old traditional texts**,** like Charaka Samhita mentioned the protective role of *Trichosanthes dioica* on important body organs like liver, spleen, heart etc, many of which are now scientifically proven. From the literature review it can be perceive that *Trichosanthes dioica* may play a significant role in developing formulations for geriatric care as it is having almost all the properties of pharmaceutical care designed for the elderly i.e. antioxidant property, antidiabetic property, cholesterol lowering, & hepatoprotective[1].

Seeds paste is used to kill worms in wounds and fungal infections[2]. The bark is used in leprosy and jaundice. Leaves of *T. dioica* have been investigated for their antioxidant[3] and anti-inflammatory activities in the past[4]. *Trichosanthes dioica* (Parwal) screened for the treatment of Alzheimer's disease[5]. Fruits of *T. dioica* reported for the hypoglycemic effect for Prevention of Type-2 Diabetes[6]. The seeds of *T. dioica* were used in treatment of helminthes ethnopharmacologically[7].

MATERIAL AND METHODS

Collection and authentication

The seeds of *Trichosanthes dioica* were collected from the neighbor village of Meerut (U.P.), and authenticated by Department of Botany, Meerut College Meerut. A voucher specimen was submitted for future reference (Ref No. MRTC/4/23/2/2014)

Pharmacognostic studies

Macroscopic characteristics

The morphological characteristics of seeds were studied and the photographs were drawn and taken with the help of Sony Corp. DSC-S980, 12.1megapixel camera. Macroscopic evaluation has been done for detailed study of morphology parameters such as colour, odor, taste, shape, size, texture and fractures were determined.

Microscopic characteristics

The microscopical studies including detailed qualitative parameters were carried out on mention part of the plant. Photographs of different magnifications were taken with Olympus Microscope, Model Olympus (India), attached to YOKO CCD Camera. Micrometric determination such as length and width of trichomes & diameter of starch grains were made for the seed part of the plant[8].

Physico-chemical parameters

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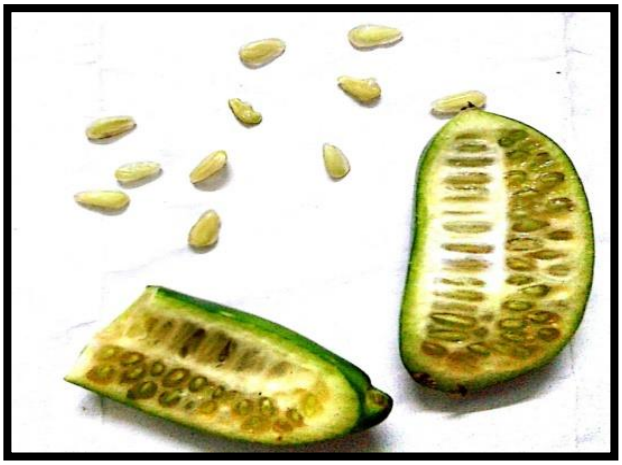


Figure 1: Photographs of fruit and seeds *Trichosanthes dioica*.

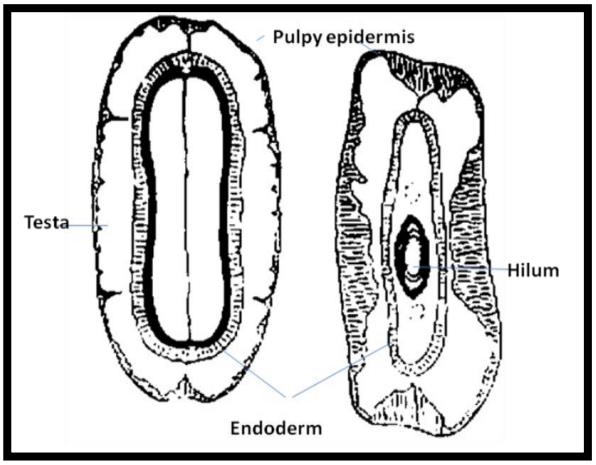


Figure 2: *Trichosanthes dioica seeds* in side view and raphe view with hilum shown as a black spot.

Table 1: Morphological characters of *T. dioica* seeds.

S. NO.	Parameters	Description of leaves
1	Colour	Yellowish white
2	Odour	Odourless
3	Taste	Characteristic
4	Shape	Ovoid-compressed
5	Size	7x5x2mm
6	Texture	Smooth

Table 2: Physicochemical parameters of *seeds* of *Trichosanthes dioica*.

Parameters	Values (% w/w)
Total Ash	4.21%*
Acid Insoluble Ash	1.32%*
Water Soluble Ash	4.29%*
Loss on Drying	24.33%*
Ether Soluble Extractive Value	16.15%*
Alcohol Soluble Extractive Value	10.11%*
Water Soluble Extractive Value	9.22%*

* n=3, (Mean value)

Table 3: Percentage yield on successive extraction in soxhlet apparatus.

Extracts	Yield (% w/w)
Petroleum ether	15.52%
Ethyl acetate	8.20%
Ethanol	7.67%

Various physico-chemical parameters as per W.H.O. guidelines have been determined for different extracts (Petroleum ether, ethyl acetate & ethyl alcohol). Some of them were Moisture content (LOD), Ash values (total ash, acid insoluble ash and water soluble ash) and extractive values (alcohol and water soluble extractive values)[9].

Phytochemical screening

Phytochemical compounds were detected in different solvent extracts (Petroleum ether, ethyl acetate & ethyl alcohol) by performing respective chemical tests. The leaves of *T. dioica* were soxhlet extracted successively with the solvent in order of increasing polarity[10]. All the extracts of the plant were subjected to phyto-chemical screening.

High-performance thin layer chromatography fingerprinting

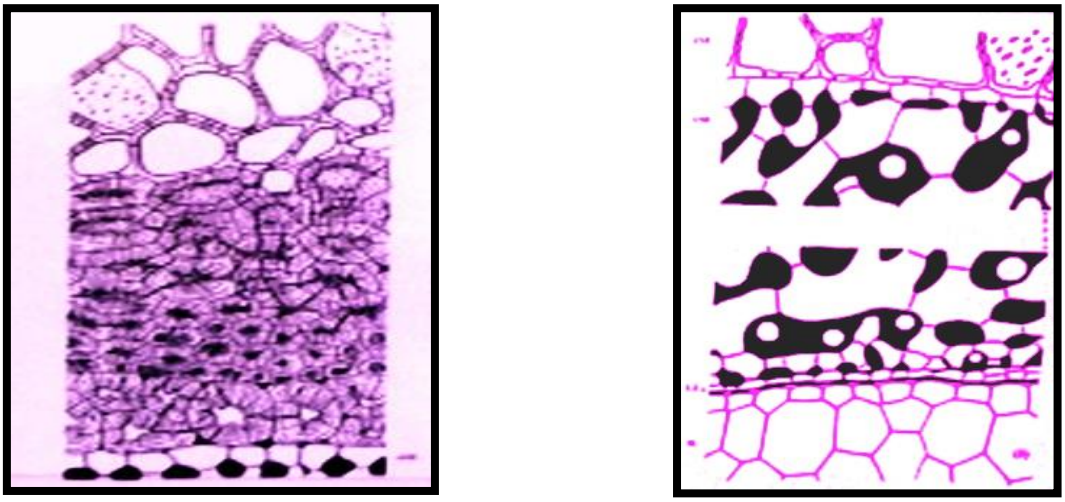
Phytoconstituents detection was done by high performance thin layer chromatography (HPTLC) of ethanol and ethyl acetate extracts in order to substantiate the standardization data of the plants. The standardization of a crude drug was an integral part of establishing its correct identity. The results of this investigation were serving as a basis for proper identification of the plant[11].

Preparation of sample solution

Ethanol and ethyl acetate extracts were used in preparation of sample solution. 5 mg/ml concentration of each extracts were prepared in respective solvents of chromatographic grade and then filtered by whatman filter paper No. 1. Prepared samples of all solvent extracts were applied on TLC aluminium sheets coated with silica gel 60 F 254 (Merck, Mumbai, India) using Linomat sample applicator.

Development of chromatograms

A number of solvent systems were tried, for ethanol and ethyl acetate extracts for better resolution and maximum number of spots, but the satisfactory resolution was obtained in the solvent Chloroform: Methanol (9:1) for ethanol extract and Hexane:Chloroform: Methanol (7:2:1) for ethyl acetate extract. The chromatograms were developed in twin trough glass chamber saturated with solvents for 20 minutes up to the distance of 80 mm. The air dried plates were viewed in ultraviolet radiation to mid-day light. Spots were visible without derivatization at 254 and 366 nm wavelengths but best results were shown when TLC plates were sprayed with detection reagent (Anisaldehyde sulfuric acid reagent and plate was heated at 110°C for 5 minutes) and then visualized in visible light range 400-600nm. Scanning was performed by CAMAG HPTLC densitometer Linomat V sample applicator equipped with a 2 µL Hamilton (USA) syringe in absorbance mode at both 254 and 366 nm, both of the extracts (ethanol and ethyl acetate) were also scanned at 350-600 nm using deuterium and tungsten lamp. The R_f values and colour of the resolved bands were noted.



a b

Figure 3: a) Outer part of mature testa,, b) Inner part of developing testa

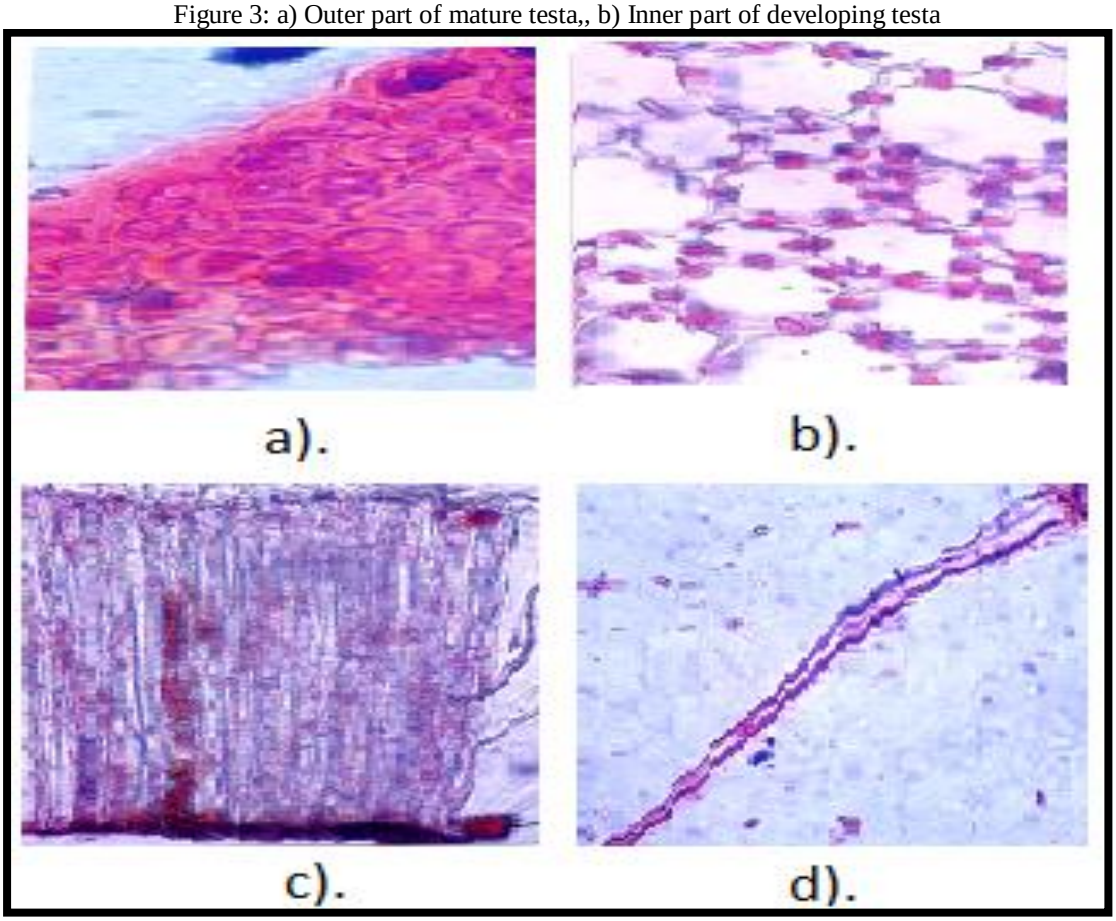


Figure 4: a) Sclerotic endodermal layer b) Innermost layer of parenchyma cells c) Mucilaginous epidermis made up of long thin trichomes d) unicellular trichome.

RESULT AND DISCUSSION

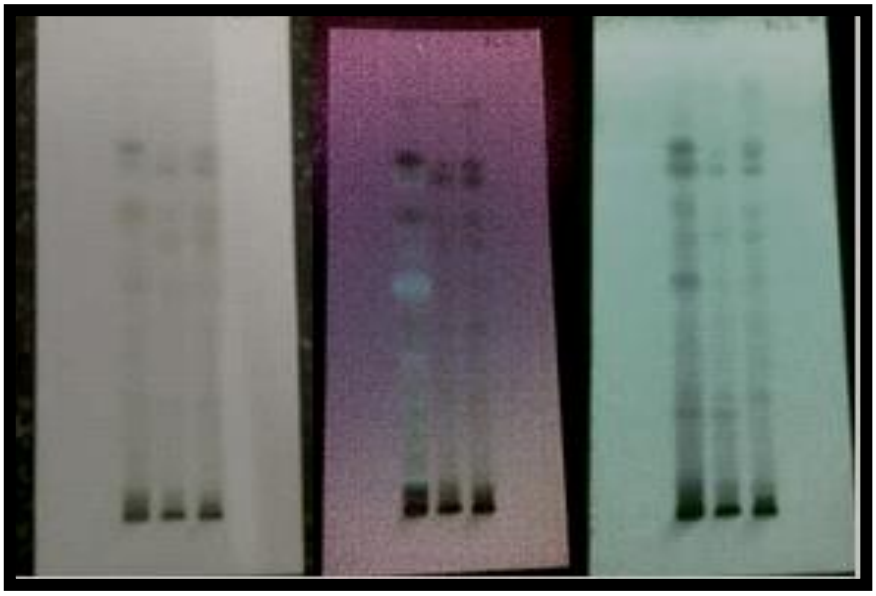
Morphology

Seeds (7x5x2mm) ovoid compressed, yellowish white, nearly smooth without pleurogram are embedded in blackish green placental pulp (Figure 1 & 2), testa is thicker along the obtuse edges.

Microscopy

Transverse Section (T.S) of seed

On staining with phloroglucinol-HCl (1:1) T.S of seed through testa, the following characteristics were observed: There was found the presence of mucilaginous epidermis made up of long thin trichomes, Innermost layer of parenchyma cells and sclerotic endodermal layer. Testa is 17-23 cells thick on the sides on the seeds. Exotesta: a layer shortly columnar pulpy cells, much elongate on the sides of micropyle thin walled but with fine fibrillar thickenings (not lignified) on the radial and inner wall, the outer wall thickened and slightly lignified, first filled with starch grains. This layer is 7-11 cells thick on the sides of seeds but more thick at the obtuse edges, composed mainly



HPTLC Plate seen at Visible light | HPTLC Plate seen at 366nm | HPTLC Plate seen at 254nm

Track 1: Ethanolic extract, Track 2: Ethyl acetate extract, Track 3: Petroleum ether extract Visible light.

Figure 5: HPTLC profile of Seeds extracts of *T. dioica.*

Table 4: Phytochemical screening of various extracts of seeds of *Trichosanthes dioica.*

S. No.	Phytoconstituents	Pet. Ether Extract	Ethyl acetate Extract	Ethanol Extract
1	Alkaloids	-	-	+
2	Glycosides	-	+	-
3	Tannins and Phenols	-	+	-
4	Flavonoids	-	+	-
5	Steroids/ Triterpenoides	-/-	-/-	-/+
6	Proteins and Amino acids	-	-	+
7	Carbohydrates	-	+	-
8	Fats and fixed oils	+	+	-

(+) = Present, (-) = Absent

Table 5: HPTLC profile of Petroleum ether Extract

Peak	Rf	Area (AU)	Area %
1	0.04	3033.3	34.52
2	0.14	1213.8	13.81
3	0.81	954.7	10.87
4	0.88	571.4	6.50
5	0.92	2860.6	32.56
6	0.94	152.7	1.74

Table 6: HPTLC profile of ethyl acetate Extract

Peak	Rf	Area (AU)	Area %
1	0.03	2596.0	17.61
2	0.09	1282.7	8.70
3	0.70	2613.7	17.73
4	0.30	1442.9	9.79
5	0.56	388.5	2.63
6	0.64	408.1	2.77
7	0.73	587.3	3.98
8	0.80	1100.9	7.47
9	0.83	388.6	2.64
10	0.86	487.9	3.31
11	0.93	3449.2	23.39

cuboidal substellate cells. Endotesta: 8-10 cells thick, thin walled without starch, aerenchymatous, substellate, the outer cell layers composed of lignified smaller cells. Tegmen disappeared except for a trace at the micropyle. Vascular bundles of raphe-antiraphe are without branches. Nucellus persistent as 2-4 cell layers with thick external cuticle.

Powder Microscopy of seed

On staining with different stains and after glycerin mounting following powder characters were observed. Ruptured sclerotesta and lignified sclerotic endodermal cells Parenchyma cells, aerenchyma cells**,** Starch gains of 41.5 µ diameter, Non lignified unicellular trichomes. Length of unicellular trichomes 0.012mm-0.2mm

Physico-chemical evaluation

Various physico-chemical parameters were analyzed by observations of three samples for each parameter and an average value of each parameter was determined as reported in Table 2.

Preliminary phytochemical screening

The successive extraction was carried out using petroleum ether, ethyl acetate and ethanol as solvents, the extract was dried using rotary evaporator and percentage yield was determined.

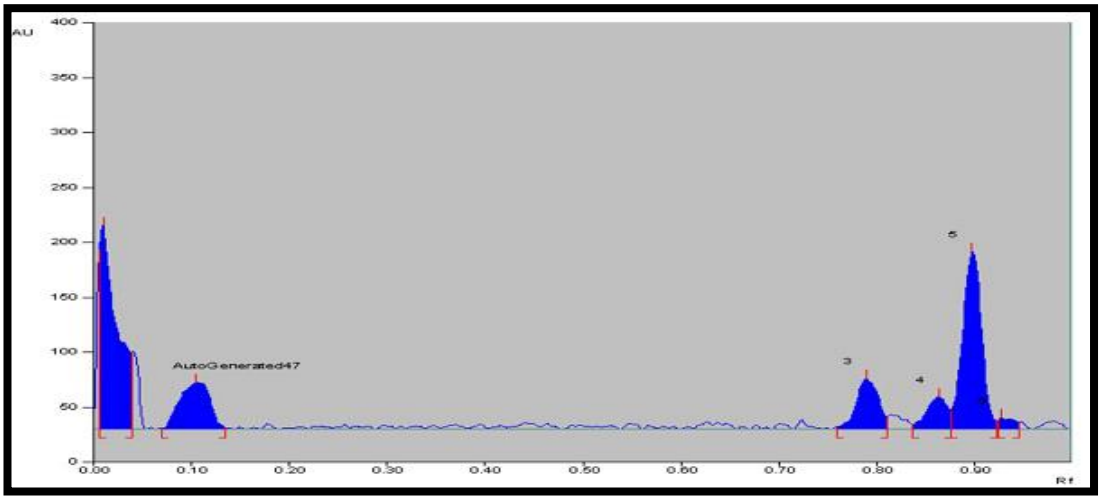


Figure 6: HPTLC chromatogram of *T. dioica* Pet. ether seeds extract showing different peaks of phytoconstituents.

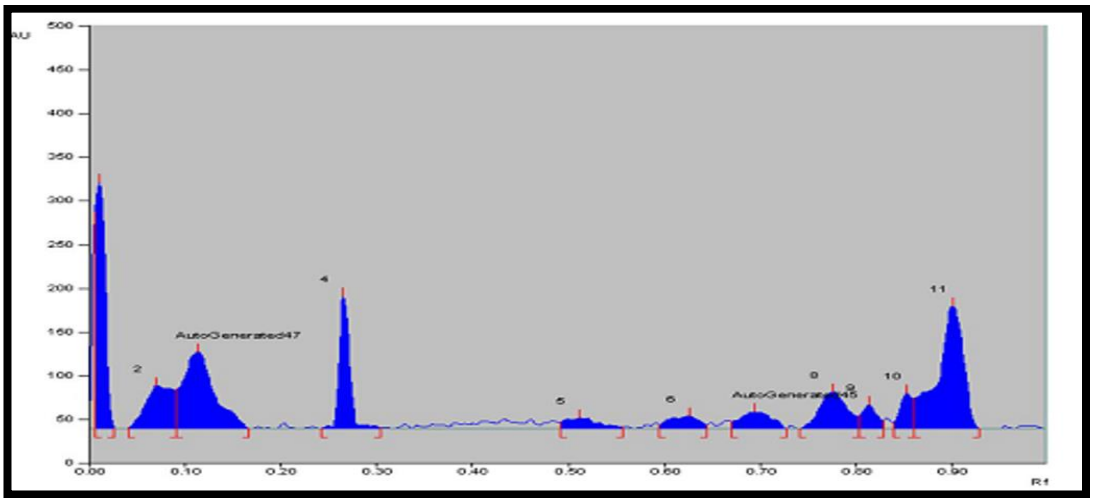


Figure 7: HPTLC chromatogram of *T. dioica* ethyl acetate seeds extract showing different peaks of phytoconstituents.

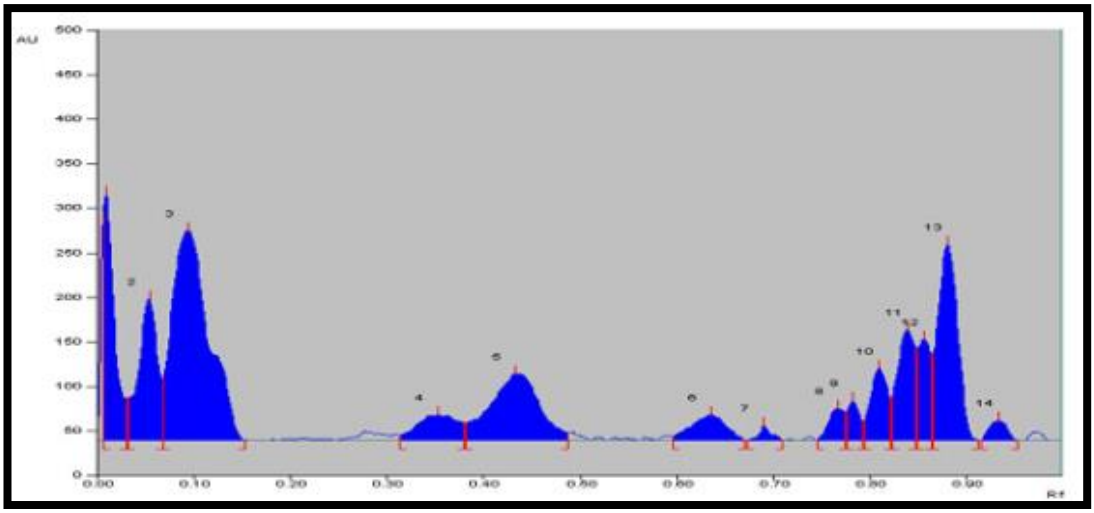


Figure 8: HPTLC chromatogram of *T. dioica* ethanolic seeds extract showing different peaks of phytoconstituents.

Preliminary phytochemical screening of all the three extracts of seeds of *Trichosanthes dioica* was performed to have an idea of the group present in the plant part.

HPTLC Profile

The results from HPTLC finger print scanned at wavelength 420 nm for Pet ether extract of *T. dioica* seeds showed six polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.04 to 0.94 in which highest conc. of the phytoconstituents was found to be 34.52% and its corresponding Rf value was found to be 0.04 respectively and was recorded in Table 5.

Table 7: HPTLC profile of Ethanol Extract.

Peak	Rf	Area (AU)	Area %
1	0.03	2853.3	9.55
2	0.07	2881.4	9.65
3	0.15	8209.2	27.48
4	0.38	1116.7	3.74
5	0.49	3472.2	11.62
6	0.67	940.6	3.15
7	0.71	174.1	0.58
8	0.78	549.1	1.84
9	0.79	479.7	1.61
10	0.82	1305.0	4.37
11	0.85	2030.5	6.80
12	0.86	1380.3	4.62
13	0.91	4114.4	13.77
14	0.95	364.3	1.22

The corresponding HPTLC chromatogram was presented in Figure 6.

The results from HPTLC finger print scanned at wavelength 420 nm for ethyl acetate extract of *Trichosanthes dioica* seeds. There are eleven polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.03 to 0.93 in which highest concentration of the phytoconstituents was found to be 23.39% and its corresponding Rf value was found to be 0.93 respectively and was recorded in Table 6. The corresponding HPTLC is presented in Figure 7.

The results from HPTLC finger print scanned at wavelength 420 nm for ethanol extract of *T. dioica* seeds. There are fourteen polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.03 to 0.95 in which highest Conc. The phytoconstituents was found to be 27.48% and its corresponding Rf value was found to be 0.15 respectively and was recorded in Table 7. The corresponding HPTLC chromatogram was presented in Figure 8.

CONCLUSION

Parwal is an important common plant, the fruit of which is an integral part of diet and consumed as a vegetable. Physico-chemical evaluation & Chemical screening of different extracts showing the occurrence of alkaloids, glycosides, Flavonoids, carbohydrates, fixed oils, steroids, tannins, phenols etc., all these findings included with HPTLC Fingerprint profile will be useful towards establishing identification, purity and quality of the herb with the specific part seeds, which is gaining relevance in herbal drug research for the identification and preparation of monograph. So the drug development from this plant through rational approach has wide scope in future.

ACKNOWLEDGMENT

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