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Full Length Research Paper

Pharmacognostic study of male leaves of *Trichosanthes dioica* Roxb. with special emphasis on microscopic technique

Saurabh Singh[1], Lalit Machawal[2]* and M. G. Chauhan[3]

[1]Department of Pharmacognosy, Faculty of Ayurveda Pharmaceutical Sciences, Lovely School of Applied Medical Sciences, Jalandhar, India.
[2]Amity Institute of Seabuckthorn Research, Amity University, Noida, Uttar Pradesh, India.
[3]Institute of Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurveda University Jamnagar, Gujarat, India.

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Today sophisticated modern research tools for evaluation of the plant drugs are available but microscopic method is one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials. Pharmacognostic investigation of the fresh, powdered and anatomical sections of the leaves of *Trichosanthes dioica* Roxb. was carried out to determine its macro- and microscopical characters and also some of its physical constants. Externally, the leaves possess a cordate base, a sinuate and dentate margin, acute to acuminate apex and both surfaces are very rough with rigid hairs surface. Internally, its shows the presences of an anomocytic stomata, unicellular, both glandular and simple covering trichomes are scattered as such throughout or attached with the cells of the epidermis. Majority of the glandular trichomes are with 4 to 5 celled uniseriate stalk and unicellular head very few are short and with uni- to bi-cellular stalk and uni- to multi-cellular head especially from that of petiole region. Simple covering multicellular uniseriate thick walled trichomes are of various sizes and usually of the cells of both simple and glandular trichomes are often embedded with cystolith. Phytochemical studies of the powdered leaves revealed the presences of alkaloids, resins, glycosides, flavonides and some carbohydrates. The pharmacognostic profile of the leaves will assist in standardization for quality, purity and sample identification.

Key words: *Trichosanthes dioica,* pharmacognostic standardization, male leaf morphology.

INTRODUCTION

Trichosanthes dioica Roxb. (Family: Curcurbitaceae) is dioecious, climber with perennial root stock. It was found wild in the plains of North India from Punjab to Assam; it is also extensively cultivated all over the warmer region of India, particularly in Uttar Pradesh, Bihar, West Bengal and Assam for its fruit (Hooker, 1973; Haines, 1961; Kanjilal, 1997). Brief description of *T. dioica* is found in the charaka samhita (Shastry et al., 1970), sushruta samhita (Acharya et al., 1980).

Leaves of the plant are considered to be rich source of bioactive compounds with many medicinal properties such as blood sugar lowering effect in experimental rat models (Chandra et al., 1988), mild diabetic human subject (Sharma et al., 1990), antifungal activity (Harit and Rathee, 1996) and antibacterial activity (Harit and Rathee, 1995). However, no work has been carried out on the male leaves of this plant, because gender variation will affect the quality, purity and pharmacognostic characters. Therefore, the present work was undertaken to study the pharmacognostic characters for the male leaves of the *T. ioica*, which could be used to prepare a monograph for the proper identification of the plant.

*Corresponding author. E-mail: lalitmachawal@gmail.com. Tel: +91-9871625491.

Table 1. Macroscopic characters of male leaves.

Characters	Male Leaf
Leaf	Simple, alternate
Shape	Ovate, oblong, cordate
Size	5 - 8 cm long; 3 - 6 cm broad
Texture	Scabrous
Apex	Acute to acuminate
Margin	Sinuate and dentate
Base	Cordate
Surface	Both surfaces are very rough with rigid hairs
Colour	Green, Dull green
Venation	Similar
Taste	Slightly bitter
Odour	Not specific
Petiole	Similar

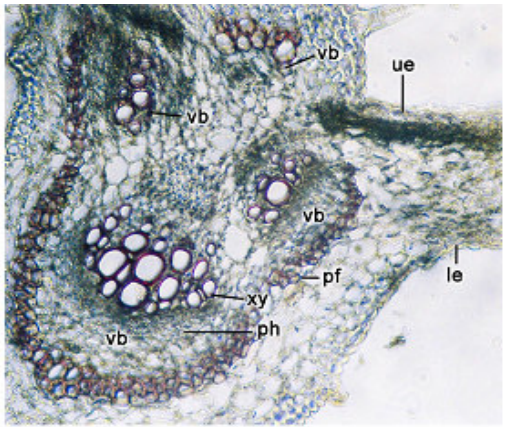

Figure 1. Detailed section of male leaf.
Col-colenchyma, cu-cuticle, glt- glandular trichome, lig b ce – lignified basal cells, pa- parenchyma, pal-palisade, ph- phloem, pf- pericyclic fibre, t- trichome, ue- upper epidermis, vb- vascular bundle, xy- xylem.

MATERIALS AND METHODS

Fresh male plants of *T. dioica* Roxb. were collected in the month of July 2005 from the cultivated field of Anand Agriculture University (Gujarat). Herbarium sheets of leaves were prepared and a voucher specimen was lodged in the museum of I. A. M. P. S. department for the future documentation. Few leaves of plants were preserved in a standard solution of (F.A.A.) (Formalin: glacial acetic acid: ethyl alcohol (70%) (10:5:85).The remaining leaf samples were dried in shade. Coarse powder (60 #) of dried leaves of male plants was stored for their microscopical study and phytochemical investigations. Free hand section was taken from preserved material for studying their microscopical characters. Leaf constants were determined from the preserved materials.

Morphology of leaf

Detailed morphology of fresh leaves of male plants of *T. dioica* Roxb. was studied and their characters are in Table 1.

Microscopic characters

T.S. of male leaves

Diagrammatic T.S. passing through the midrib projects strongly at the lower side and elevated at the upper side, 3 to 4 meristels of various sizes encircled by the sclerenchymatus band lie in the centre and lamina is dorsiventral. It bears simple uniseriate and multicellular and glandular trichomes with unicellular stalk and multicellular head and with multicellular stalk and unicellular head, embedded with yellowish contents. The simple trichomes are straight or bent or often resting on a pedestial base and also are embedded with cystolith. Collenchymatous tissue lies underneath both the upper and lower midrib region, it being 1 to 2 layered only, at the lateral sides of the lower midrib region, bicollatral meristele of various sizes lies in the centre, the lowest being bigger in size and is composed of radially arranged xylem vessels associated with trachids, fibers and medullary rays. Except the upper most meristele lying underneath the collenchymatous tissue all other meristele are encircled by a continuous band of sclerenchyma fibers (Figure 1). Lamina is composed of upper and lower epidermis with cuticle and bear Trichomes. Under the upper epidermis lies a layer of palisade cells, the remaining cells of the mesophyll are consisting of 3 to 4 rows of spongy parenchyma traversed with obliquely cut vascular bundle.

T. S. of male petiole

Diagrammatic T. S. of the petiole is irregularly oval to somewhat rectangular in shape, with highly pubescent margin and show a discontinuous ring of vascular bundles in the peripheral region of ground tissue. Detailed T.S. of the petiole shows, layer of epidermis with thick cuticle and plenty of simple and glandular trichomes just like that of leaf. 5 to 10 rows of collenchymatous hypodermis lies underneath the epidermis, the remaining ground tissue being parenchymatous and is traversed with rings of bicollateral vascular bundles. The group of parenchymatous cells lying in between the vascular bundles and few cells of the pith becomes lignified in male leaves.

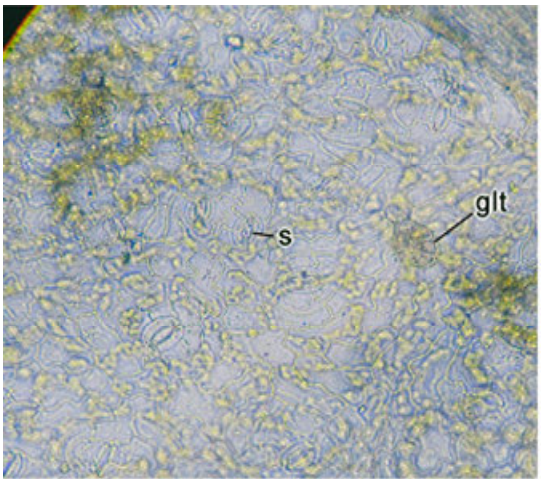

Figure 2. Upper epidermal cells, showing stomata.

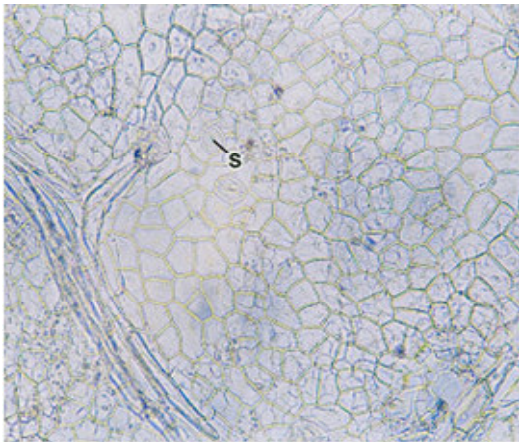

Figure 3. Lower epidermis cell, showing stomata.

Surface preparation of male leaves

Surface preparation of male leaves shows few anomocytic stomata, cicatrix and glandular trichomes in the upper epidermis (Figure 2). Lower epidermis of leaves show wavy anti clinical walled cells with plenty of anomocytic stomata, glandular trichomes with unicellular stalk and multicellular club shaped head are common (Figure 3).

More details of microscopic characters of male leaves shows the following characters

Upper and lower epidermis in surface view often exhibiting cicatrix cells of the upper epidermis are much more bigger in size than that of lower, with straight anti clinical walls. Lower epidermal cells are smaller in size with slightly wavy anti clinical walls and are embeded with anomocytic stomata. Both glandular and simple covering trichomes are scattered as such throughout or attached with the cells of the epidermis. Majority of the glandular trichomes are with 4 to 5 celled uniseriate stalk and unicellular head very few are short and with uni- to bicellular stalk and uni- to multicellular head especially from that of petiole region. Simple covering multicellular uniseriate thick walled trichomes are of various sizes and usually of the cells of both simple and glandular trichomas are often embedded with cystolith. Epidermal cells at places are papilose and often exhibit some buldgings of the glandular leaf teeth. Transversely cut fragments of the lamina showing a layer of palisate lying underneath the epidermis and few cells of the mesophyll embaded with very few prismatic crystals of calcium oxalate. Longitudinally cut spiral and few pitted vessels and lignified and non lignified fibers of the meristele and wide lumen pitted groups of sclereids from midrib and petiole.

Table 2. The physiochemical constants of male leaves.

No.	Parameters	Male
1.	Foreign matter, % w/w	-
2.	Loss of drying at 105℃, % w/w	7.8
3.	Ash value, % w/w	13.36
4.	Acid insoluble ash, % w/w	3.14
5.	Water soluble extractive, % w/w	12.5
6.	Alcohol soluble extractive, % w/w	2.1
7.	pH of 10% w/v aqueous solution of leaves	7.65

Quantitative microscopic characters of the male leaves

The male leaves studied for determining the various leaf constants and general parameters are as follows:

Stomata index
Upper epidermis 4.5 - 5.8
Lower epidermis 12 - 13
Vien-islets number 3 - 4
Palisade ratio 3.5 - 4

Organoleptic characters of the leaves

Organoleptic characters like colour and taste of male leaves are colour of the leaves was green, taste slightly bitter and without any characteristic odour.

Physiochemical investigation (The Ayurvedic Pharmacopoeia of India, Pharmacopoeia Standards of Ayurvedic formulations, 1987)

The physiochemical constants of male leaves are entered in Table 2.

Qualitative chemical tests

Male leaves were subjected to the following preliminary tests; the detection of the presence of various constituents like alkaloids (Stephen, 1965), flavonoids (Peach and Tracy, 1955), cucurbitacins (Egan, 1969), resin etc.. Estimation of total alkaloids found to be 0.25% w/w the percentage of total alkaloid was calculated (Stephen, 1969).

T. L. C. of alkaloids

Concentrated methanolic extracts of the leaf was subjected to chromatographic separation of alkaloids using Silica gel G as a

Table 3. Under long ultra violet.

No.	Male leaf	
	R_f	Colour
1	0.44	Pinkish brown
2.	0.65	Orange
3.	0.76	Orange
4.	0.86	Orange

Table 4. After spraying Dragendroff's.

No.	Male leaf	
	R_f	Colour
1	0.44	Pinkish brown
2.	0.65	Dark green
3.	0.76	Light green
4.	0.86	Brown

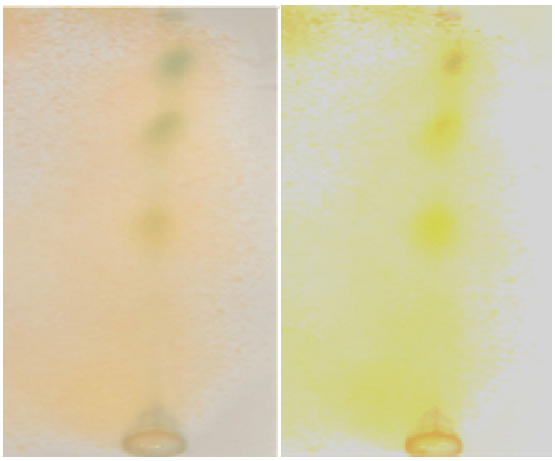

Figure 4. TLC profile, long uv (366 nm) derivatized, TLC profile of methanolic extract of *Trichosanthes dioca*, solvent system: toluene: ethyl acetate: diethylamine (7:2:1) spray reagent Dragendoff's.

stationary phase and toluene: ethylacetate: diethylamine (7: 2: 1) as a mobile phase. The solvent was allowed to run up to 19 cm. The plate was observed as such under ultra-violet light. R_f values of number of fluorescent resolved spots were calculated and entered in Table 3. The plate later on was sprayed with Dragendorff's reagent and R_f value of different colored spots resolved were calculated and entered in Table 4 and Figure 4.

HPTLC finger printing

1 g of leaf powder separately was successively macerated thrice with 10 ml methanol in 50 ml stopper conical flask by keeping it for one night. The pooled extracts were evaporated under reduced pressure, the weight of dried extract was noted down and then it was dissolved in 2 ml of methanol and was subjected for the HPTLC separation (Figure 5).

Gas chromatography

In this order first of all preparative chromatography of alcoholic (concentrated) extract was done by using general solvent system of alkaloid, toluene: ethylacetate: diethylamine (7: 2: 1). Then solvent was allowed to run up to appropriate height, the spots were marked with needle and silica powder of these spots was carefully scraped and collected separately and dissolved in methanol. The gas chromatogram of the above prepared sample reveals 4 peaks having retention time 3.79, 14.59, 35.35 and 36.57 the peak corresponding to sample is the one having retention time 35.35 and 36.57 mm. Other peaks are representing some impurity/ contamination of the column. On further analyzing the compound emerging for GC column in mass spectrometer the two probable compound identified were:

(1) Ferrocene, 1, 1", 3,3' - Tetrakis (1, 1-dimethylethyl) with molecular formula $C_{26}H_{42}Fe$ and molecular weight of 410
(2) 1,1' 2',1" - Terphenyl, 3',4' - dimethyl - 5',6' – diphenyl with molecular formula $C_{32}H_{26}$ and molecular weight of 410

Spectral analysis (IR)

Looking to the spectral study of the newly synthesized (dihydropyridines) molecules, the carbonyl (> C=O) of the amidic functionality stretching was observed at 1655 - 1670 cm^{-1} and another ketonic group (ester, or acetyl) was observed at approximately at 1670 - 1690 cm^{-1} due to the conjugation with the DHP skeleton. The amide (> C-N stretching) isobserved at 1300 - 1400 cm^{-1}. The stretching of secondary amine (> NH) appears approximately around 3100 - 3400 cm^{-1} which indicates the presence of -NH_ in the compound. Here in almost all the compounds two -NH sis vibration are observed for since there are two secondary amine groups. One of them shows absorption at lower frequencies due to the carbonyl group attached to it. The stretching C-N appears at 1200 -1400 cm^{-1} which further adds up the evidence of the presence of secondary amine group. *T. dioica* peak obtain from I.R. spectrum. Indicate the methyl overtone, amide and aromatic functional groups etc.

RESULTS AND DISCUSSION

To detect the quality of any plant whether it is dioecious or monoecious (male and female flowers on same plant). Standardization is essential measure for quality, purity and sample identification. Microscopic method is one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials. Various exogenous and endogenous factors have been taken in to account for determining the quality and purity of plant. *T. dioica* Roxb. named as Patoli, Jyotsna and Patola. *Trichosanthes dioica* commonly name as Patola Patra is one of the highly reputed drug used in Ayurveda and is incorporated in number of Ayurvedic formulations, irrespective for their gender. Male leaves bear simple uniseriate and multicellular and glandular trichomes with unicellular stalk and multicellular head and with multicellular stalk and unicellular head. Collenchymatous

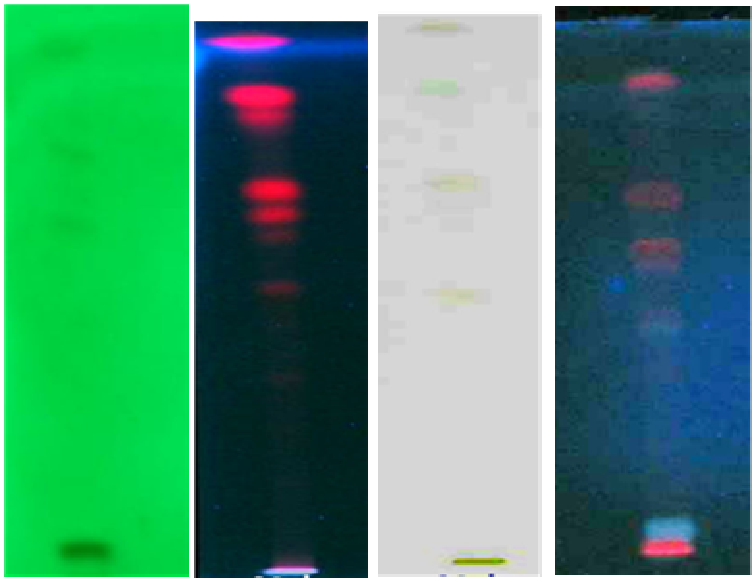

Figure 5. HPTLC plates, short uv 254 nm Long uv 366 nm day light derivatized HPTLC profile of metanolic extract with solvent system: toluene: ethyl acetate: diethylamine.

various sizes lies in the centre, the lowest being bigger in size and is composed of radically arranged xylem vessels associated with trachids, fibers and medullary rays. Except the upper most meristele lying underneath the collenchymatous tissue of all other meristele are encircled by a continuous band of sclerenchyma fibers. Surface of male leaves shows few anomocytic stomata, cicatrix and glandular trichomes in the upper epidermis. Lower epidermis with plenty of anomocytic stomata, glandular trichomes with unicellular stalk. Chemical tests detection of the presence of alkaloids, flavonoids, cucurbitacins and resin. Hence it was thought worth to investigate pharmacognostic profile of the leaves will assist in standardization for quality, purity and sample identification.

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