

**PHARMACOBOTANICAL STUDIES ON ‘SHVET SHARPUNKHA’ – A
COMPARATIVE DIAGNOSTIC ACCOUNT OF TEPHROSIA VILLOSA
PERS. AND T. PURPUREA (LINN.) PERS. FORM ALBIFLORA S. R.
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ABSTRACT: Two kinds of ‘sharpunkha’, the ‘Shvet’ (white) and ‘Rakta’ (red) are described in some of the Ayurvedic texts and the former is reported therapeutically more effective. Some of the Ayurvedic physicians use *T. villosa* Pers. as ‘Shvet sharpunkha’ due to its persistently villous silky white parts. While others have advocated white colour of flowers as main feature for distinguishing “Shvet sharpunkha”. A white flowered form of *Tephrosia purpurea* which is found in association with red or purple flowered ones is reported by us as *T. purpurea* (Linn.) Pers. Form *albiflora* S. R. Paul et R. C. Gupta. In the present work, however, detailed comparative pharmacognosy of all vegetable parts of *T. villosa* and *T. purpurea* f. *albiflora* have been carried out. Also the study reveals that two species exhibit great similarity in their macro – an microscopical feature.

INTRODUCTION

“Sharpunkha”, an important Ayurvedic drug, has been in use for a long time. Although it has not found a mention in *Charka Samhita* and ‘Kalshak’, described there in, is attributed to *Corchorus capsularis* Linn, (Charak. 1949), its first applications seems to be in an anti-rabies preparation as reported in *Sushrut Samhita* (Sushrut, 1952). Three other references pertaining to curative uses are also available in *Astang Hridayam*, the third great classic work of Ayurvedic (Vagbhata, 1950).

In *Nighantus*, the drug is highly valued for its anthelmintic, antipyretic, alternative and coagulant properties. It is also recommended in liver, spleen and heart troubles and vitiation of ‘vata’ (Bhavamisra, 1949; Madanpal, 1903; Narahari, 1933).

Several such synonyms as ‘Nilvikshakriti’ (indicates resemblance to *Indigofera tinctoria* Linn.): ‘Sharvidha’. ‘Banpunkha’, ‘Isupunkha’ (Mean the pinion of an arrow in allusion to the triate venation in its leaves); ‘Sitpunkha’, ‘Shubhrapunkha’, ‘Shvetpunkha’ (implies silky white downy leaves); ‘Kalshak’, ‘Kalaka’, ‘Shraddh shak’ (refer to vegetable used during the *Shraddha* ceremonies) and ‘Pleehshatru’ ‘Pleehar’ (ascribes its curative application in spleen troubles) etc., denote this drug (Bhavamisra, 1949; Godbole *et al*, 1966; Madan Pal, 1903; Narahari, 1933; Vagbhata, 1950), and unanimously attributed to the plant species *Tephrosia purpurea* (Linn.) Pers. of family Leguminosae (Kirtikar and Basu, 1933; Sharma, 1956).

Two kinds of ‘Sharpunkha’, the ‘Shvet’ (white) and the ‘Rakta’ (red) are described

in some of the Ayurvedic texts and the former is reported therapeutically more effective (Chunekar, 1969; Narahari, 1933; Sharma, 1956; Vagbhatta, 1950). It has been regarded by some that *Tephrosia purpurea* is the 'Rakta Sharpunkha' has come to be known so, for its red flowers (Chunekar, 1969; Nadkarni, 1954; Sharma, 1956; Vagbhatt, 1950). Another species, *Tephrosia villosa* pers. too has pale-red flowers. It is, therefore, not clear whether the colour of flowers has been the basis for the differentiation of two kinds or some other diagnostic feature. According to present day Ayurvedic physicians the 'Shvet Sharpunkha' is *Tephrosia villosa* due to its persistently villous silky white parts (Chunekar, 1969; Sharma (1956). Sharma (1956) and Singh and Chunekar (1972) have, however, advocated white colour of flowers as main feature for distinguishing 'Shvet Sharpunkha'. This view receives support also from *Shivdatta Nighantu* which mentions: "Sharpunkheti vikhyata shvetpushpa kvachid bhavet".

During the course of our studies a white flowered form of *Tephrosia purpurea* was collected from suburbs of Lucknow and described as *Tephrosia purpurea* (Linn.) Pers forma *albiflora* S. R. Paul et R. C., Gupta (Gupta and Paul, 1978). A detailed chemical analysis and clinical trial of this complex may reveal the identity of genuine 'Shvet Sharpunkha'. The present work, however, includes a detailed comparative diagnostic account of vegetative parts of *Tephrosia villosa* and *T. purpurea* f. *albiflora*.

MATERIALS AND METHODS

Root, stem and leaves of *Tephrosia purpurea* f. *albiflora* at various growth stages were collected in August – September from suburbs of Lucknow and dried, and

preserved plant materials of *T. villosa* collected in September-October from various localities of Andhra Pradesh were procured. Hand sections of plant material were employed for histological studies. Isolated elements were studied after macerating the material with Schultz's fluid. The ash values, extractive percentage according to I. P. method (Anonymous, 1966) and fluorescence analysis was performed according to Kokoski *et al* (1958). Behaviour of powdered drug on treating with different chemical reagents was noted. The stomatal index, palisade-ratio, vein-islet and vein-termination number were determined according to Wallis (1967) Hotch (1948) from the leaves treated with dilute nitric acid followed by clearing in chloral hydrate. Preliminary phytochemical studies and TLC pattern of extractives on silicagel G were performed using usual methods and histochemical tests as per Kay (1938) and Johansen (1940).

RESULTS AND DISCUSSION

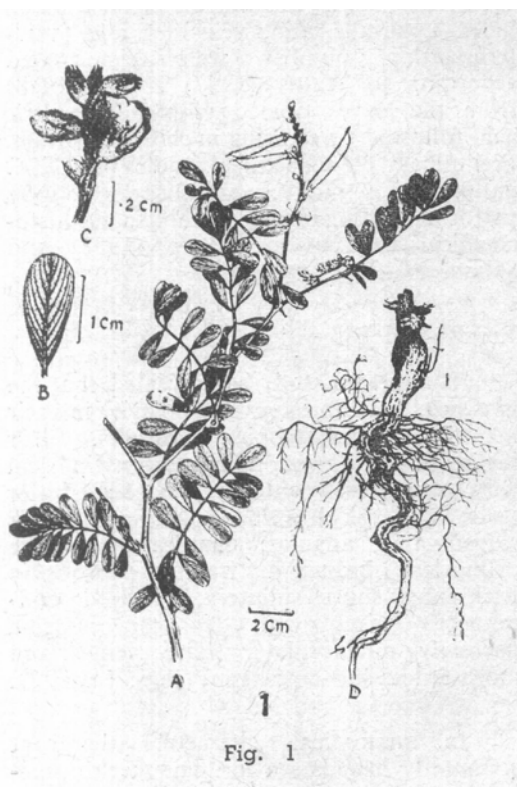
The present study has revealed that the two species exhibit a great similarity in their macro-and microscopical features. For instance: both have long taproots cylindrical with transverse lenticels and a bitter taste; the apical part of young stem is ridged and angular, mature stems are cylindrical, branched, fracture fibrous in bark and short splintery in wood; odd- pinnate leaves with leaflets opposite, narrowly oblanceolate, triate veined and more or less subcoriaceous (Figs. 1 and 2).

In anatomical characters the root commonly has diarch primary stele, phelogen arises in the pericycle, fibres develop at an early stage (Fig. 3A-D) and secondary phloem occupies a wider zone with wide rays forming funnel-shaped dilations towards its outer region, phloem fibres occur solitary

and in small groups intermixed with usual phloem cells; root has diffused porous wood and its conjunctive tissue differentiated into highly lignified and partially lignified zones (Fig 4 A-D). In young stem of two species trichomes are both glandular and non-glandular, (Figs. 13 a-b and 14 A-C), chlorenchymatous cells occur under the furrows of assimilatory young stem, secretary canals are found in cortex and pith and phellogen originates more commonly in outer cortex (Figs. 5A-B and 6 A-B). In periderm, pericycle and phloem of mature stem of the two species the individual elements are more or less alike in form and appearance but vary in dimension, xylem is highly lignified diffused porous with vessels commonly isolated and in radial groups of 2-4; xylem fibres occupy the major part of the wood (Figs. 7 A-B and 8 A-B). Starch grains and calcium oxalate crystals are more or less alike (Figs. 13c, 14d). In leaf of two species the common microscopic characters are: occurrence of anisocytic stomata along

with few other types (Figs. 11 A-B and 12 A-B), embedded and transcurrent veins, crystal-sheath surrounding the large vascular bundle which are capped by fibre strands on both adaxial and abaxial sides (Figs. 9A-B, 10A-B), collenchymatous central ground tissue and pericycle in basal pulvinus region of rachis as well as in petiolule and a complex and highly characteristic series of changes of vascular system in its course through the rachis (Figs. 15 a1 - a3 and 16 A-C).

However, various macro- and microscopic characters coupled with such physicochemical observations as the behaviours of drug powders with different chemical reagents and ultraviolet radiation, percentage-extractives and ash values and preliminary phytochemical studies including chromatographic pattern of extractives exhibit remarkable differences (Table I - VII).



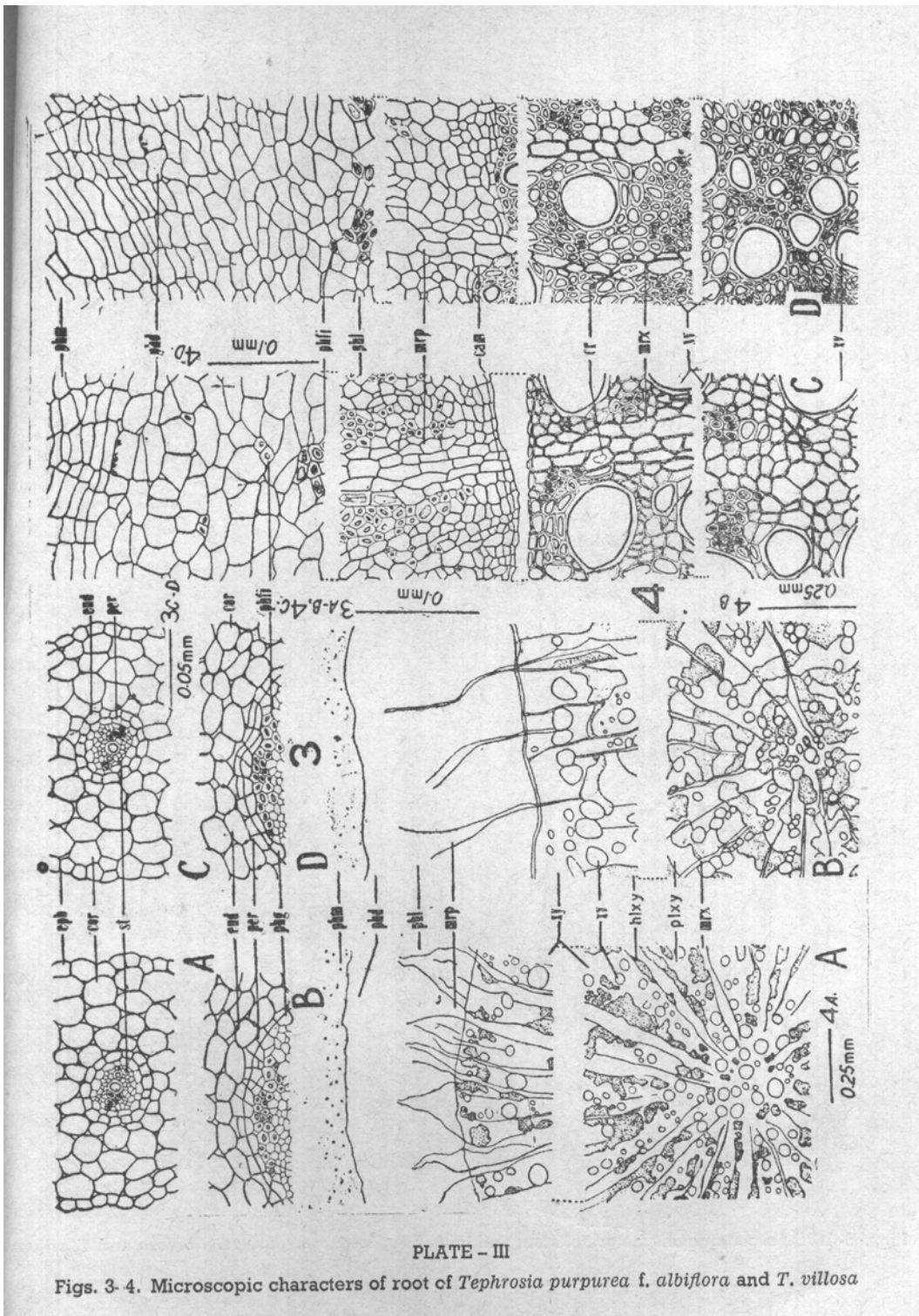


PLATE - III

Figs. 3-4. Microscopic characters of root of *Tephrosia purpurea* f. *albiflora* and *T. villosa*

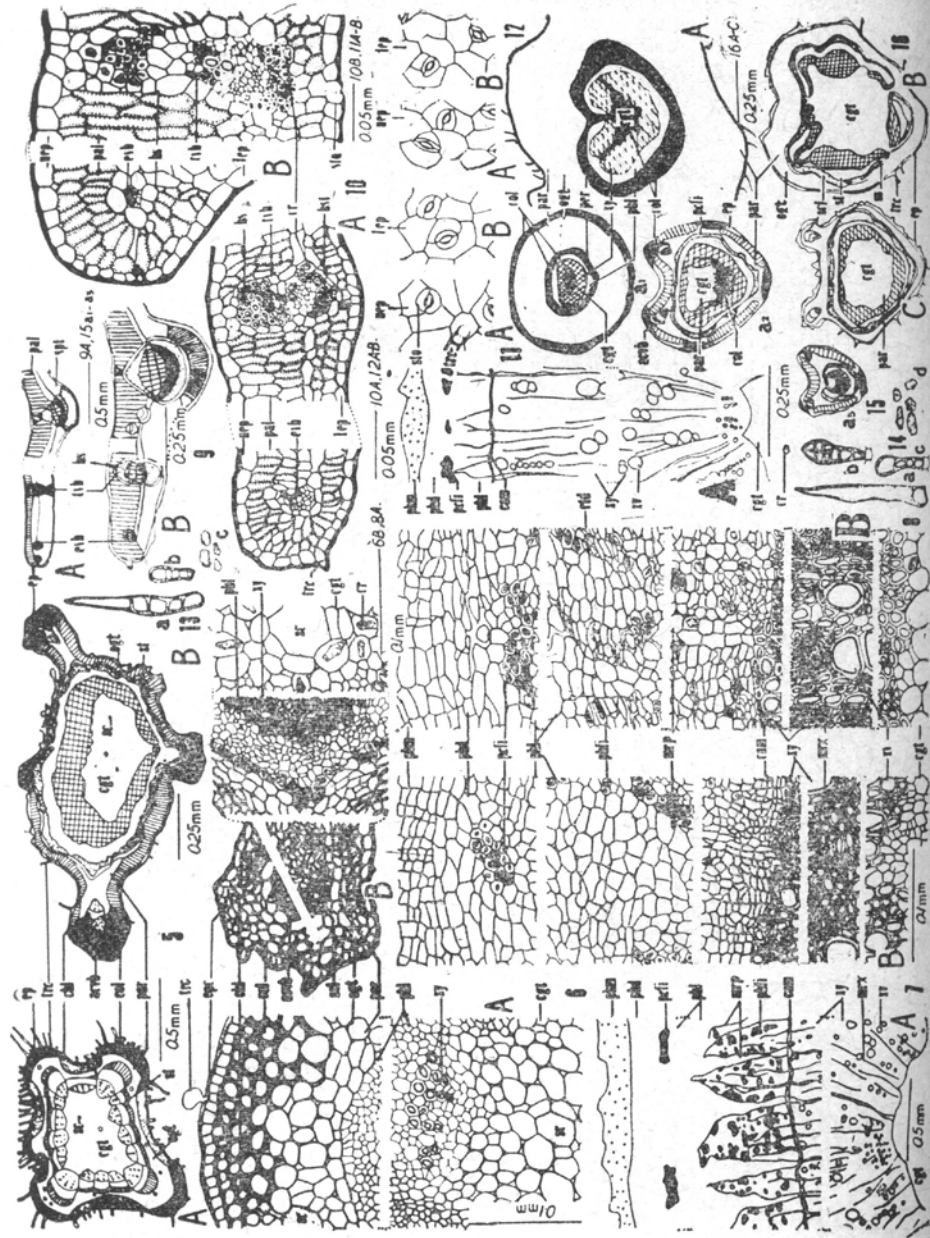


PLATE - IV

Figs. 5-16. Microscopic characters of stem and leaf of *Tephrosia purpurea* f. *albiflora* and *T. villosa*

TABLE – I

Distinguishing organoleptic characters of *Tephrosia villosa* and *T. purpurea* form *albiflora*

Plant Parts	Characters	<i>Tephrosia villosa</i>	<i>Tephrosia purpurea</i> <i>form albiflora</i>
1	2	3	4
<i>Root</i>	Colour	Yellowish brown to brick – brown	Pale – white
	Taste	Bitter	Bitter, slightly tingling
<i>Stem</i>	Surface	Densely hairy with white or grayish depressed hairs, glabrous in lower mature parts	Usually glabrous, pubescent only towards the apex
<i>Leaf</i>	Rachis – size	4 – 8 cm	6 – 10 cm
	Leaflet		
	(A) number	9 – 17	5 – 13
	(B) size	1.2 – 2 x 0.5 – 1 cm	2 – 2.8 x 0.5 – 1.2 cm
	(C) surface	Almost glabrous above, silky beneath	Almost glabrous above, pubescent beneath

LEGEND TO FIGURES

PLATE – I

Figure 1. Macroscopic characters of *Tephrosia purpurea* f. *albiflora*

A: a twig, B: a leaflet, C: flowers, D: a piece of root.

PLATE – II

Figure 2. Macroscopic characters of *Tephrosia villosa*

A: a twig, B: a leaflet, C: fruit, D: a piece of root.

PLATE – III

Figures 3 – 4. Microscopic characters of root of *Tephrosia purpurea f. albiflora* and *T. villosa*.

3. A. a part of t.s. of young root of *T. purpurea f. albiflora* showing cellular details
- B. a part of t.s. of young root of *T. purpurea f. albiflora* showing formation of phellogen and phloem fibre.
- C. a part of t.s. of young root of *T. villosa* showing cellular details.
- D. a part of t.s. of young rot of *T. villosa* showing formation of phellogen and phloem fibres.
4. A. a part of t.s. of mature root of *T. purpurea f. albiflora* (diagrammatic).
- B. a part of t.s. of mature root of *T. villosa* (diagrammatic).
- C. a part of t.s. of fig 4 A. showing cellular details.
- D. a part of t.s. of fig.4 B. showing cellular details.

PLATE – IV

Figures 5 – 16. Microscopic characters of stem and leaf of *Tephrosia purpurea f. albiflora* and *T. villosa*

5. A. t.s. (diagrammatic) of a young stem of *T. purpurea f. albiflora*.
- B. t. s. (diagrammatic) of a young stem of *T. villosa*.
6. A.: a part of t.s. of fig 5A. showing cellular details
- B. : a part of t.s. of fig 5B. showing cellular details
7. A : a part of t.s. of mature stem of *T. purpurea f. albiflora* (diagrammatic)
- B : a part of t.s. of fig 7A. showing cellular details.
8. A.: a part of t.s. of mature stem of *T. villosa* (diagrammatic)
- B.: a part of t.s. of fig 8A. Showing cellular details.
9. A: t.s. (diagrammatic) through a portion of leaflet of *T. purpurea f. albiflora*.
- B. : t.s. (diagrammatic) through a portion of leaflet of *T. villosa*.
10. A: a part of t.s. of fig. 9A. Showing cellular details.
- B.: a part of t.s. of fig. 9 B. showing cellular details.

11. Surface view of leaflet of *T. purpurea f. albiflora*.
- A : upper epidermis showing *anomocytic* stomata and a portion of trichome.
 - B. : lower epidermis showing *anomocytic* stomata.
12. Surface view of leaflet of *T. villosa*.
- A. : upper epidermis showing *anomocytic* and *anisocytic* stomata
 - B. : lower epidermis showing *anisocytic* stomata
13. A: covering trichome.
- B: a glandular trichome
 - C: prismatic crystals of calcium oxalate.
14. a : a typical covering trichome.
- b-c: glandular trichomes
 - d: prismatic crystals of calcium oxalate
15. t. s. through various portions of rachis of *T. purpurea f. albiflora* (diagrammatic)
- a 1: proximal basal portion of pulvinus
 - a 2: distal region of first internodes of rachis.
 - a 3: extreme distal portion of rachis.
16. t.s. through various portions of rachis of *T. villosa* (diagrammatic)
- A: proximal basal portion of pulvinus.
 - B: distal region of first internode of rachis
 - C: extreme distal portion of rachis.

TABLE – II
Distinguishing microscopical characters of root of *Tephrosia villosa* and *T. purpurea* form *albiflora*

Plant parts	Characters	<i>Tephrosia villosa</i>	<i>Tephrosia purpurea</i> <i>form albiflora</i>
<p><i>Young root</i> <i>Mature root</i></p>	<p>Primary stele Phloem rays – A. Uni-to biseriate B. Multiseriate Xylem rays – A. Uni – to biseriate B. Multiseriate Xylem parenchyma – Distribution Size of different cells in macerations A) Phloem fibres B) Crystal fibres C) Vessel elements D) Tracheids E) Xylem fibres F) Xylem parenchyma</p>	<p>Di-triarch More commonly 6 – 14 cells high (rays 2 – 20 cells high) 3 – 5 cells wide; More commonly 14 – 17 cells high (rays 9 – 26 cells high) More commonly 8 – 16 cells high (rays 2 – 24 cells high) 3 – 6 cells wide; More commonly 19 – 38 cells high (rays 6 – 55 cells high) Occur in paratracheal aliform confluent to regular broad bands and occasionally terminals 8-35 x 550-1750 µm 13-26.5 x 152-435 µm 13-91 x 65-200 µm 8-18 x 65-240 µm 8-21 x 346-1716 µm 10-77 x 13-87 µm</p>	<p>Diarch More commonly 9 – 16 cells high (rays 6 – 24 cells high) 3 – 6 cells wide; More commonly 20 – 30 cells high (rays 6 – 57 cells high) More commonly 5 – 9 cells high (rays 1 -12 cells high) 3 - 7 cells wide; More commonly 20 - 28 cells high (rays 10 – 42 cells high) Occur predominantly in narrow wavy or strait bands or broken lines between rays and touching and partially enclosing the pores and rarely being aliform confluent. 8-18 x 164-473 µm 6-22 x 84-200 µm 10-220 x 18-360 µm 8-22 x 160-360 µm 8-13 x 220-1150 µm 10-25 x 50-225 µm</p>

TABLE – III
Distinguishing microscopical characters of stem of *Tephrosia villosa* and *T. purpurea* form *albiflora*

Plant parts	Characters	<i>Tephrosia villosa</i>	<i>Tephrosia purpurea</i> <i>form albiflora</i>
<i>Young stem</i>	Out line in t.s.	Shows gradual development of two wing like expansion on laterals and one ridge in middle in lower younger region of stem	No such development
	Trichomes – (A). Glandular	40 – 62 µm long with 2 – 4 celled stalks and club shaped uni – to bicellular head	30 – 50 µm long with 1 – 3 celled stalks and multi-cellular globose to elliptic head
	(B) Non-glandular	148 – 1151 µm, uniseriate, 3 – 5 celled	127 – 623 µm long and typically 2 – celled
	Accessory bundles	Three (Two in lateral wings and one in middle ridge)	Absent
<i>Mature stem</i>	Phloem rays – A. Uni-biseriate	More commonly 8 – 16 cells high (rays 4 – 22 cells high)	More commonly 6 – 12 cells high (rays 4 – 18 cells high)
	B. Multi-seriate	3 – 6 cells wide; More commonly 16 – 20 cells high (rays 10 – 32 cells high)	3 – 9 cells wide; More commonly 20 – 32 cells high (rays 5 – 60 cells high)
	Ray cells-size in l. s. Xylem parenchyma	12 – 42 x 16 – 49 µm Being unilateral paratracheal to occasionally vasicentric and aliform	38 – 52 x 8 – 78 µm Occur in diffused short tangential aggregates, touching and partially enclosing the pores and are rarely vasicentric to aliform-confluent
	Size of different xylem cells in macerations		
	A. Vessel elements	25 – 99 x 82 – 313 µm	35 – 140 x 18 – 220 µm
B. Tracheids	16 – 25 x 115 – 395 µm	10 – 35 x 80 – 280 µm	
C. Xylem fibre	8 – 30 x 296 – 740 µm	8 – 20 x 290 – 820 µm	

TABLE – IV
Distinguishing microscopical characters of leaf of *Tephrosia villosa* and *T. purpurea* form *albiflora*

Plant parts	Characters	<i>Tephrosia villosa</i>	<i>Tephrosia purpurea</i> <i>form albiflora</i>
1	2	3	4
Raghis Leaflet	<p>A. Outline in t.s.</p> <p>B. Trichome</p> <p>Epidermis</p> <p>A. Adaxial epidermal cells size in t.s</p> <p>B. Abaxial epidermal cells size in t.s</p> <p>C. Stomata</p> <p>Histological quantitative study</p> <p>A. Stomatal index</p> <p>(i) On adaxial surface</p> <p>(ii) On abaxial surface</p> <p>B. Palisade ratio</p> <p>C. Vein-islet number</p> <p>D. Vein-termination number</p>	<p>Shows a pair of small wing like adaxial ridge</p> <p>10 – 24 x 12 – 45 μm</p> <p>12 – 16 x 16 – 24 μm</p> <p>Mixed anomocytic and anisocytic</p> <p>17.88, with range of variation 16 – 18</p> <p>20.06, with range of variation 17 – 25</p> <p>6.83, with range of variation 4 – 9</p> <p>21.666, with range of variation 18 – 25</p> <p>30.111, with range of variation 25 - 35</p>	<p>Adaxial ridge not so prominent</p> <p>Non-glandular, typically 2 – celled</p> <p>20 – 40 x 20 – 60 μm</p> <p>12 – 20 x 14 – 36 μm</p> <p>Usually anisocytic and paracytic occasionally anomocytic</p> <p>15.8, with range of variation 11 – 24</p> <p>12.4, with range of variation 6 – 16</p> <p>4.38, with range of variation 3 – 6</p> <p>23.28, with range of variation 14 – 29</p> <p>24.00, with range of variation 12 - 33</p>

TABLE – V
Distinguishing ergastic contents, extractive – percentage and ash values of *Tephrosia villosa* and *T. purpurea* form *albiflora*

Characters	<i>Tephrosia villosa</i>	<i>Tephrosia purpurea</i> form <i>albiflora</i>
Cell – contents		
A. Prismatic crystals – size	6 – 17 x 13 – 52 μm	4 – 16 x 6 – 18 μm
B. Starch grains – size	6 – 14 x 4 – 12 μm	4 – 10 μm in diameter
C. Plant bases	Absent	Present
Extractive – percentage (I.P.)		
A. Alcohol sol. Extract	11. 256	4 .63
B. Water sol. Extract	7. 256	15. 135
Ash values (I.P.)		
A. Total ash (%)	5.42	10.558
B. Acid in sol. Ash (%)	0.473	3.308

TABLE – VI
Behaviour of powders of *Tephrosia villosa* and *T. purpurea* form *albiflora* with different chemical reagents and ultra – violet radiation

Treatments	Behaviour	
	<i>Tephrosia villosa</i>	<i>Tephrosia purpurea</i> form <i>albiflora</i>
Effect of chemical reagents on powders		
A. Aqueous extract + 4% NaOH + 1% CuSO ₄ soln. heated	Bluish green precipitate	Orange precipitate
B. Aqueous extract + Resorcinol + Conc. HCl heated	No change	Turned dark red
C. Aqueous extract + Phloroglucinol + Conc. HCl	No change	Turned reddish orange

Fluorescence analysis of Powders		
A. Powder + Nitrocellulose in amylacetate		
Root	Fluoresces pale – brown	Pale – green
Stem	Fluoresces brownish pale-green	Dull pale brown
Leaf	Fluoresces bright orange	Scarlet red
B. Powder + 50% Nitric acid		
Root	Fluoresces dark green	Olive green
Stem	Fluoresces orange with slight green tinge	Greenish orange
Leaf	Fluoresces brownish green	Orange with slight green tinge

TABLE – VII
Chromatographic pattern of *Tephrosia villosa* and *T. purpurea* form *albiflora* under ultra-violet

Plant parts	Extractives	Chromatographic Pattern	
		<i>Tephrosia villosa</i>	<i>Tephrosia purpurea</i> form <i>albiflora</i>
Root	Petro. Ether extractive developed with solvent system benzene-chloroform (10:20)	Of the 17 components, the major one (hRf 49.0) fluoresces bluish green	Of the 17 components the major one (hRf 49.0) fluoresces violet-blue
	Chloroform extractive developed with solvent system benzene-chloroform-acetone (8:30:2).	Of the 15 components none fluoresces violet blue	Of the 21 components two major ones (hRf 75.4 and 89.8) and one minor (hRf 57.7) fluoresce violet – blue
Stem	Petro. Ether extractive developed with solvent system benzene-chloroform (10:20)	Of the 16 components the major one (hRf 38.1) fluoresces bluish green	Of the 12 components the major one (hRf 23.6) fluoresce red
Leaf	Petro ether extractive developed with solvent system benzene – chloroform (10:20)	Of the 16 components five (hRf 12.7, 20.9, 31.8, 43.6 and 98.1) fluoresces bluish green	All the 5 components (including trailings) Fluoresce pinkish red

Abbreviations used

Acvb, accessory vascular bundle; bs, bundle sheath; bst, bundle sheath extention; cam, cambium; cgt, central ground tissue; chl, chlorenchyma; col, collenchyma; cor, cortex; cr, calcium oxalate crystal; end, endodermis; ep, Epidermis; epb, epiblema; epc, epidermal cell; evb, embedded vascular bundle; hlxy, highly lignified xylem; lep, lower epidermis; mrp, phloem ray; mrx, xylem ray; ogt, outer ground tissue; pal, palisade cells; par, parenchyma; pefi, pericyclic fibre; per, pericycle; phd, phelloderm; phfi, phloem fibre; phg, phellogen; phl, phloem; phm, phellem; plxy, partially lignified xylem; rtd, rhytidoma; sc, secretary canal; scl, sclerenchyma; spt, spongy tissues; cs, crystal sheath; st, stele; sto, stomata; trc, trichome; tvb, transcurrent vascular bundle; uep, upper epidermis; xy, xylem; xv, xylem vessel.

REFERENCES

- Charak: *Charak Samhita*, Jamnagar, *Sutra* – 27/91, *Chikitsa* – 13 / 182 (1948).
- Sushrut: *Sushrut Samhita*, Kanpur, *Kalpa* – 7, (1952).
- Vagbhata : *Astang Hridayam*, Varanasi, *Sutra* – 6/97, *Chikitsa I* 15/85, *Uttar* – 30/26, (1950).
- Bhavamisra : *Bhava Prakash*, Varanasi – Gayaghat, pp. 204 – 5, (1949).
- Madanpal: *Madanpal Nighantu*, Bombay, pp. 54, (1903).
- Narahari: *Raj Nighantu*. Calcutta, pp. 71 – 74, (1933).
- Godbole, S. R.; Pendse, G. S. and Bedekar, V. A. : *Glossary of vegetable drugs in Vagbhata*, Poona, pp. 208 (1966).
- Kirtikar, K. R. and Basu, B. D. : *Indian Medicinal plants*, Allahabad, 1, 734 – 36, (1933).
- Sharma, P. V. : *Dravyaguna Vigyan*, Varanasi, 2 – 3, 440 – 42, (1956).
- Chunekar, K. C., *Bhava Prakash Nighantu of Bhavamisra*, Varanasi, pp. 408, (1969).
- Nadkarni, A. K. : *Dr. K. M. Nadkarni's Indian Materia Medica*, Bombay, pp. 561 – 63, (1954).
- Singh, B. and Chunekar. K. C. : *Glossary of vegetable drugs in Brhattravi*, Varanasi, pp. 391, (1972).
- Gupta, R. C. and Paul, S. R.: A white flowered form of *Tephrosia purpurea* (Linn.) Pers. (Leguminosae) Natl. Acad. Sci. Lett. 1 (2), 47 – 48, (1978).
- Anonymous: *Pharmacopoeia of India*, New Delhi, pp. 947 – 48, (1966).

Kokosi, C.J. : Kokoski, R. J and Slama F. J.: Fluorescence of powdered vegetable drugs under ultraviolet, J. Amer. Pharm. Assoc. (Sci. Edn.) , 47 (10), 715 – 17, (1958).

Wallis, T. E. : *Text book of Pharmacognosy*, London, pp. 113 – 16, (1967).

Hotch, J. H. : Fifty years of Quantitative Microscopy in Pharmacognosy, Econ. Bot. 2: 111, (1948).

Kay, A. L. : *Microscopical studies of drugs*, London, pp. 18, (1938).

Johansen, D. A. : *Plant micro-technique*. New York, (1940).