



## Comparative foliar micro morphological studies on some *Tephrosia* Pers. species of Indian Thar Desert

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### ABSTRACT

A comparative foliar micro morphological study of four species of *Tephrosia* Pers. viz. *Tephrosia purpurea*, *Tephrosia uniflora* subsp. *petrosa*, *Tephrosia villosa*, *Tephrosia wallichii* of Indian Thar desert was carried out to elucidate their taxonomic relationship. Stomata and trichomes along with epidermal cell characters were investigated for demarcation of species. Epidermal cells were polygonal isodiametric and rectangular in shape with straight to curved anticlinal wall. All four species were amphistomatic and in addition to classical stomatal types, some new stomatal types such as staurocytic, isotricytic, hemiparacytic, anomotetracytic, brachyparateracytic were also reported. Trichomes were two types- simple, uniseriate, eglandular and peltate glandular and occurred on lower surface of leaf in all four species. Trichomes were present on upper epidermis in *T. villosa* and *T. wallichii* but absent in *T. purpurea*, *T. uniflora* subsp. *petrosa*. Present study on foliar epidermis of all four species of *Tephrosia* revealed that all micro morphological characters of leaf shows inter specific relationship among the species, which could be remarkable and reassuring taxonomic tool in delimitation of these species.

**Key words:** *Tephrosia*, Indian Thar Desert, stomata, trichomes, epidermal cell

### INTRODUCTION

The Indian Thar desert is a large arid region which occupied about 9 per cent of the Indian and 61 per cent of Rajasthan geographical area. This lies between 68° to 71° E longitude and 24° to 28° N latitude on the western part of Rajasthan. The boundary on east is delimited by Aravalli hills, on west it forms natural boundary with Pakistan, on the north Bikaner and Nagaur district are situated and on the south it extends up to Kutch of Gujarat state. Most of the area of this desert comprises of very dry part. This region is characterized by extreme temperature dry and hot high velocity wind and uneven rain fall. The rainy season comes during month of July to September.

The soil is loose and sandy, saline, chalky and alkaline. The genus *Tephrosia* commonly known as "Wild indigo" belongs to family Fabaceae distributed in tropical and subtropical regions of the world. It comprises of about 400 species (Mabberley, 2005). About 29 taxa with 27 species, one variety and one subspecies were already reported from India (Kumar and Sane, 2003). Ten species were reported from Rajasthan (Singh and Shetty, 1987) and eight species of *Tephrosia* were described from Indian Thar desert region (Bhandari, 1978). Most of the species of this genus are herb and grow in wasteland and on rocks as weed. Leaves are stipulate, imparipinnate rarely simple. Flowers are zygomorphic and hermaphrodite with five sepals and five petals. Plants of this genus are used in treatment of various diseases like asthma, dropsy, rheumatic pains, stomach ache, respiratory disorders and inflammation etc. (Dalwadi *et al.*, 2014). It is also used as green manure and cover crop of crop plantation.

In present study four species of *Tephrosia* viz. *Tephrosia purpurea* (Linn.) Pers., *Tephrosia uniflora* Pers. sub sp. *petrosa* (Blatt. and Hallb.) Gillett and Ali, *Tephrosia villosa* (Linn.) Pers. and *Tephrosia wallichii* Graham were selected. *T. purpurea* is an erect, branched perennial herb with more or less hairy stem and leaf opposed or terminal raceme inflorescence. *T. uniflora* sub sp. *petrosa* is a sub fruticose and profusely branched herb found on rocky habitat. Branches are thin, angular; pubescent and arise from the base. Flowers are mostly solitary axillary or often geminate. *T. villosa* is an erect under shrub with white tomentose branches and striate stem. Flower fascicled or paired in spicate raceme. *T. wallichii* is an erect shrub with woody stem and spreading branches. Inflorescence is a terminal raceme with 3-4 flowers together at each node but inflorescence length is shorter than *T. purpurea*.

Foliar epidermis offers valuable taxonomic information in distinguishing various taxa. Systematic significance of epidermal characters has been discussed by various workers. (Rajgopal, 1979; Leelavathi and Ramayya 1983; Zarinkamar, 2007; Essiett *et al.*, 2012; Shokefun *et al.*, 2014). Microscopic features of leaf epidermis for taxonomic study includes trichomes, epidermal cells, stomata and subsidiary cells. All these epidermal characters help in identification, rearrangement and delimitation of taxa on every level. The object of present study was to provide detailed micro morphological information and a complimentary data to make easy identification of species on basis of micro morphology.

In addition to this it would be helpful in delimitation and assessment of the taxonomic relationships among the species studied.

## METHODOLOGY

The fresh and fully expanded mature leaves were collected for micro morphological study. The plant material collected from various location of Jodhpur, Jaisalmer and Barmer districts of Rajasthan, covering a large area of the Thar Desert. The specimens collected were authenticated by taxonomist of Botanical survey of India except *T. wallichii* which was identified with the help of herbarium specimen of Department of botany, Jai Narain Vyas University, Jodhpur. The voucher specimens of four species were deposited in the herbarium of department of botany of the same university.

Leaves were washed with fresh water to remove dust substances. Leaf surface was gradually scrapped off with the help of a sharp razor blade. Epidermal peels obtained from adaxial and abaxial surfaces were gently cleaned with the help of a soft camel hair brush and watered until the loose cells were washed away from peels. The epidermal peels were stained with one per cent aqueous Saffranine and rinsed in water to remove excess stain and then mounted in ten per cent glycerin on a clean glass slide and covered with cover slip. Good microscopic preparations were investigated and photographed under microscope attached with Nikon FX-35A Japan camera. Measurement of epidermal cells, trichomes and guard cells were made with the aid of an ocular micrometer and finally converted by ocular constant according the power in which they were taken. Ten microscopic fields were selected randomly. All measurement except the length of trichomes was taken on uniform magnification (450X) from both surfaces. Length of trichomes was taken on 100x. The stomatal index was calculated according to Metcalfe and Chalk (1979), using the formula:

$$\text{Stomatal Index (S.I.)} = \frac{S}{S+E} \times 100$$

Where S = Number of stomata per unit area

E = Number of epidermal cells in the same area

## RESULTS

The cumulative account of foliar epidermal characters of four species of *Tephrosia* was shown in Table 1, 2, 3 and fig. 1, 2, 3, 4. In the present study descriptive

terminology was adapted from combination of stomatal classification scheme of Pant (1965), Dilcher (1974) and

Wilkinson (1979). Foliar epidermal study revealed a number of important micro morphological characters.

**Table 1. Leaf epidermal Characteristics of *Tephrosia* Pers. species.**

Char. Species	Leaf Surface	Cell shape	Wall pattern	Stomatal Type	Stomatal Density	Trichomes Density
<i>T. purpurea</i>	Adaxial	Polygonal Rectangular isodiametric	Straight curved	Ani, Ano, IT,H,Lat, BP Par, Sta, BPT, A.T	9-10	Absent
	Abaxial	Polygonal Rectangular isodiametric	Straight curved	Ani, Ano, IT, H,Lat, BP Par, Sta, BPT, AT	8-10	2-3
<i>T. uniflora</i> sub sp . <i>petrosa</i>	Adaxial	Polygonal Rectangular isodiametric	Straight curved	Ani, Ano, IT, H, Lat, BP Par, Sta, BPT, AT	15-18	Absent
	Abaxial	Polygonal Rectangular isodiametric	Straight curved	Ani, Ano, IT, H, Lat, BP Par,Sta, BPT, AT	11-13	1-2
<i>T. villosa</i>	Adaxial	Polygonal Rectangular isodiametric	Straight curved	Ani, Ano, IT, H, Lat, BP Par,Sta, BPT, AT	20-22	2-3
	Abaxial	Polygonal Rectangular isodiametric	Straight curved	Ani, Ano, IT,H,Lat, BP Par, Sta, BPT, AT	14-15	3-5
<i>T. wallichii</i>	Adaxial	Polygonal Rectangular isodiametric	Straight curved	Ani, Ano, IT, H, Lat, BP Par,Sta, BPT, A.T	18-20	2-4
	Abaxial	Polygonal Rectangular isodiametric	Straight curved	Ani, Ano, IT, H, Lat, BP Par,Sta, BPT, AT	15-17	5-8

**Abbreviations:** Ani- Anisocytic, Ano-Anomocytic,IT-Isotricytic, H- Hemiparacytic, Lat- Laterocytic, BP- Brachyparacytic, Par-Paracytic, Sta-Staurocytic, BPT- Brachyparatetracytic, AT- Anomotetracytic

**Table 2. Trichomes characteristics of *Tephrosia* (Pers.) species.**

Char Species	Leaf surface	Long trichome		Short trichome		Peltate glandular trichome	
		Length (Mean±SE) µm	Width (Mean±SE) µm	Length (Mean±SE) µm	Width (Mean±SE) µm	Length (Mean±SE)µm	Width (Mean±SE) µm
<i>T. purpurea</i>	Adaxial	-	-	-	-	-	-
	Abaxial	597.18 ±38.03	17.16 ±0.64	170.82 ±10.24	13.0 ±0	23.4 ±0.82	22.36 ± 1.32
<i>T. uniflora</i> sub sp. <i>petrosa</i>	Adaxial	-	-	-	-	-	-
	Abaxial	648.24 ±60.25	16.64 ±0.64	188.7 ±14.47	11.96 ±0.64	23.4 ± 0	21.32 ± 0.52
<i>T. villosa</i>	Adaxial	-	-	145.92 ±8.88	7.8 ±0	24.96 ±0.64	23.4 ±0.82
	Abaxial	730.38 ±48.56	14.04 ±1.04	119.88 ±13.77	9.88 ±0.52	20.28 ± 0.97	19.76 ± 0.64
<i>T. wallichii</i>	Adaxial	-	-	173.28 ±21.14	10.4 ±0	26.0 ±0.82	24.44 ±1.04
	Abaxial	517.26± 24.72	10.92 ±0.52	155.04 ±16.98	8.32 ±0.52	24.44 ±0.64	21.84 ±0.64

Note: Results based on mean of three replicates.

**Table 3. Epidermal cell and stomatal characteristic of *Tephrosia* (Pers.) species.**

Char. Species	Leaf surface	Number of epidermal cell	Epidermal cell length (Mean±SE) µm	Epidermal cell width (Mean±SE) µm	Guard Cell Length (Mean±SE) µm	Guardcell width (Mean±SE) µm	Subsidiary cell length (Mean±SE) µm	Subsidiary cell width (Mean±SE) µm	Stomatl Index (%)
<i>T. purpurea</i>	Adaxial	73.33	43.68 ±2.52	24.96 ±1.4	23.4 ±0	7.8 ±0	37.87 ±2.95	12.78 ±1.20	14.56 ±0.60
	Abaxial	66.66	47.84± 2.55	17.16 ±0.64	24.96 ±0.64	9.36 ±0.64	45.51 ±2.30	13.83 ±0.91	15.74 ±0.39
<i>T. uniflora</i> sub sp. <i>petrosa</i>	Adaxial	82.66	35.88 ±4.14	19.76 ±1.94	20.36 ±0.44	5.2 ±0	28.86 ±1.92	15.4 ±2.40	10.48 ±0.09
	Abaxial	76.66	40.56 ±2.27	21.32 ±2.08	20.8 ±0	7.28 ±0.52	32.5 ±2.46	16.64 ±2.88	15.66 ±0.52
<i>T. villosa</i>	Adaxial	134.66	30.68 ±2.52	15.08 ±0.97	16.64 ±1.04	5.2 ±0	51.48 ±1.42	20.28 ±0.61	16.78 ±0.41
	Abaxial	120.33	29.64 ±2.41	17.68 ±1.27	18.2 ±0.82	7.8 ±0	28.6 ±1.40	26.52 ±1.12	17.03 ±0.53
<i>T. wallichii</i>	Adaxial	115.66	33.8 ±1.16	24.44 ±1.33	17.76 ±0.64	6.76 ±0.64	28.6 ±1.02	13.1 ±1.22	16.76 ±0.94
	Abaxial	80.33	31.72 ±2.23	16.64 ±1.32	21.32 ±0.52	7.8 ±0	34.84 ±1.04	9.36 ±0.54	15.60 ±0.42

Note: Results based on means of three replicates.

**Epidermal cells:** The epidermal cells were polygonal, rectangular or isodiametric with straight to curved anticlinal wall pattern on both surfaces in all species (Fig. 1A&B; 2A&B; 3A&B; 4A&B). Epidermal cell size varied in all species, highest epidermal cell size was recorded in lower epidermis of *T. purpurea* (47.84±2.55 × 17.16±0.64 µm). Smallest size of epidermal cell was observed in lower epidermis of *T. villosa* (29.64±2.41 × 17.68±1.27 µm). Highest number of epidermal cells per unit area was observed in *T. villosa* (120.33) and lowest number was in *T. purpurea* (66.66). Number of epidermal cells were higher on upper epidermis in all the species. *T. villosa* and *T. wallichii* have higher number of epidermal cells per unit area as compared to *T. purpurea* and *T. uniflora* subsp. *petrosa*. Epidermal cell size and number was more on upper epidermis.

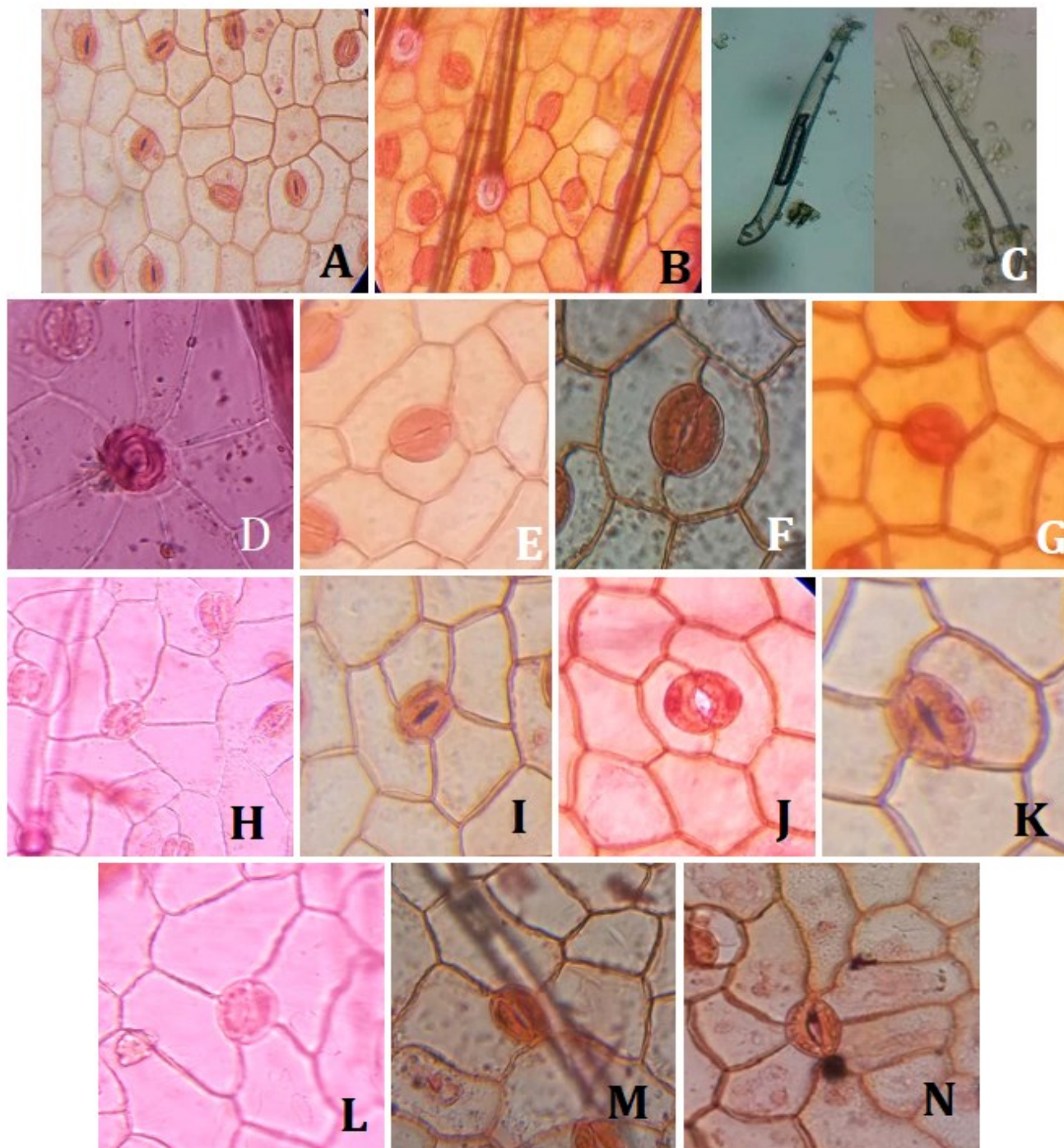
**Stomata:** Leaves were amphistomatic, stomata found on both the surfaces. Mature stomatal types were anisocytic (Fig.1E, 2E, 3E, 4E); paracytic ( Fig. 1F, 2F, 3F, 4F); isotricytic (Fig. 1G, 2G, 3G, 4G); brachyparacytic (Fig. 1H, 2H, 3H, 4H); brachyparatetracytic (Fig. 1I, 2I, 3I, 4I); laterocytic (Fig. 1J, 2J, 3J, 4J); hemiparacytic (Fig.1K, 2K, 3K, 4K); staurocytic (Fig. 1L, 2L, 3L, 4L) and anomotetracytic (Fig. 1M, 2M, 3M, 4M), anomocytic (Fig. 1N, 2N, 3N, 4N). Anisocytic, laterocytic and paracytic-were frequently found in all species on both the surfaces. Anomocytic stomata were more on lower epidermis than upper epidermis in all species.

Staurocytic, brachyparacytic and brachyparatetracytic and hemiparacytic were partially distributed on both the surfaces of leaf in all four species. Highest stomatal density was reported on the upper epidermis of *T. villosa* and it was 20-22 stomata per unit area and lowest was in 8-10 in upper epidermis of *T. purpurea*. The highest guard cell length (24.96±0.64µm) was observed in of *T. purpurea* where as it was lowest (16.64±1.04µm) in *T. villosa*. Highest guard cell width (9.36±0.64µm) was also reported in *T. purpurea* and lowest (5.2±0 µm) was reported in *T. villosa* and *T. uniflora* subsp. *petrosa* both. The highest stomatal index (16.78± 0.41µm) was reported in upper epidermis of *T. villosa* where as it was lowest (10.48±0.08µm) were found in upper epidermis of *T. uniflora* sub sp . *petrosa*.

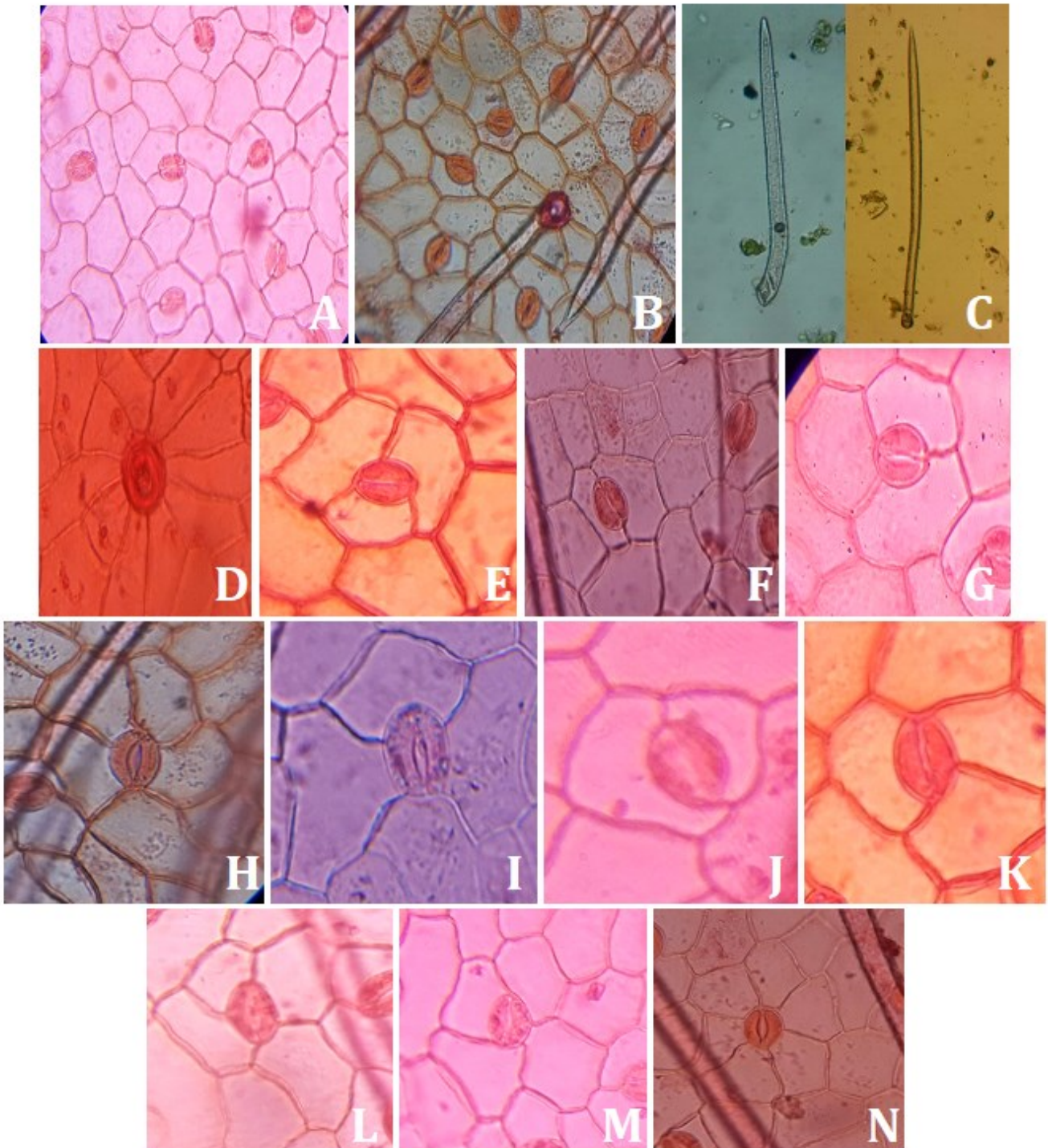
**Trichomes:** Trichomes characters considered in the present study were their size, shape, density and occurrence of trichomes on both the surfaces of leaf. Trichomes were of two types- simple non glandular (Fig. 1C, 2C, 3C, 4C) and peltate glandular (Fig. 1D, 2D, 3D, 4D). In all species eglandular trichomes were present on lower surface. In *T. purpurea* and *T. uniflora* sub sp. *petrosa*, they were absent on upper surface of leaf, while in *T. villosa* and *T. wallichii* they were present on both surfaces with variations in density and size. Trichomes of upper surface were shorter in length and comparatively less dense than lower epidermis. On the basis of length – long trichomes and short trichomes

were reported on lower epidermis. These were densely distributed on the margins of leaves. The longest trichomes ( $730.38 \pm 48.56 \mu\text{m}$ ) and shortest trichomes ( $119.88 \pm 13.77 \mu\text{m}$ ) were observed on lower epidermis of *T. villosa*. Highest trichomes width ( $17.16 \pm 0.64 \mu\text{m}$ ) was reported in *T. purpurea* where as it was lowest

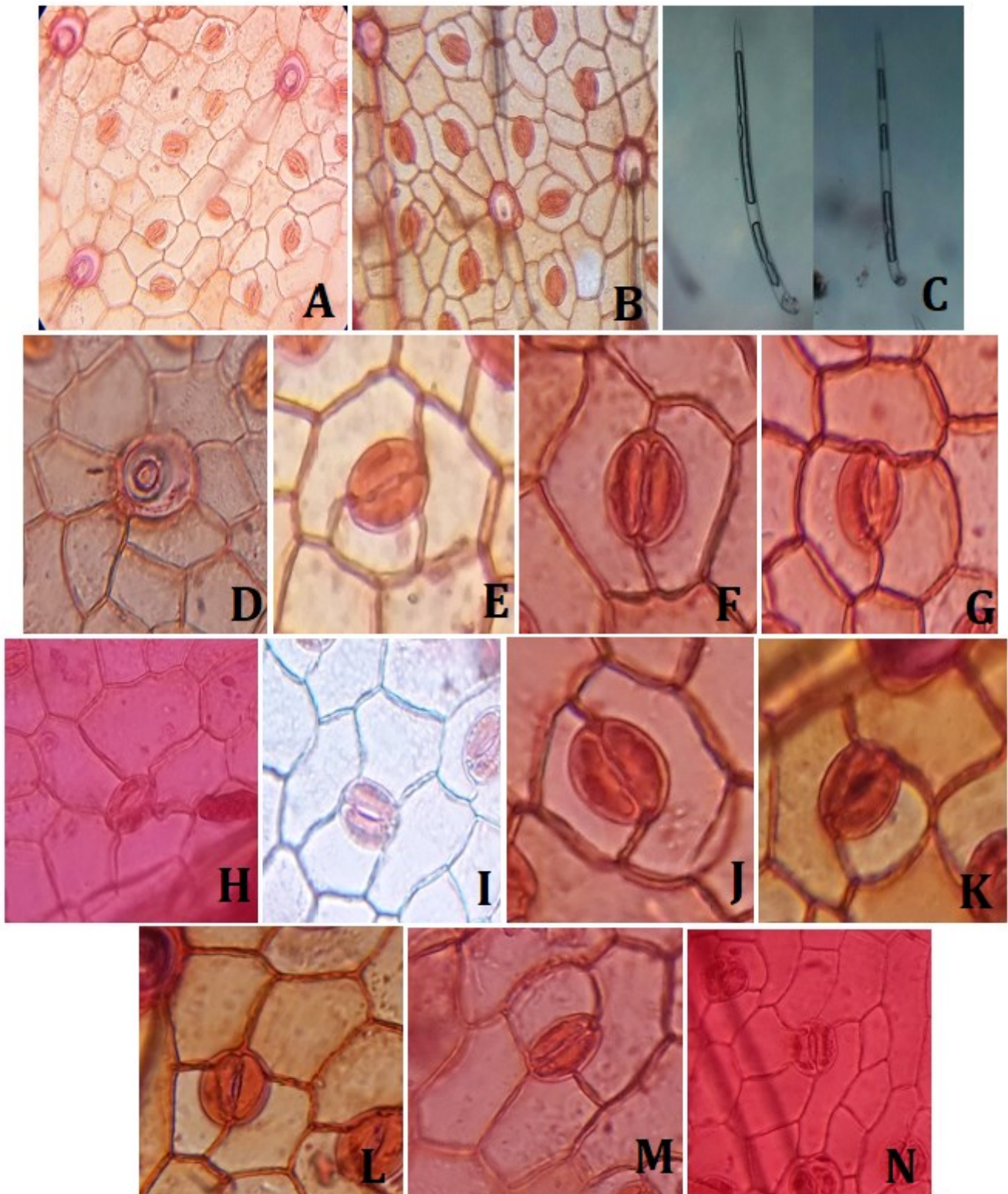
width of trichomes appear in ( $7.8 \pm 0 \mu\text{m}$ ) in *T. villosa*. Highest trichomes density (5-8) was found in lower epidermis of *T. wallichii*. Lowest trichomes density (1-2) was reported on lower epidermis of *T. uniflora* sub sp. *petrosa*.



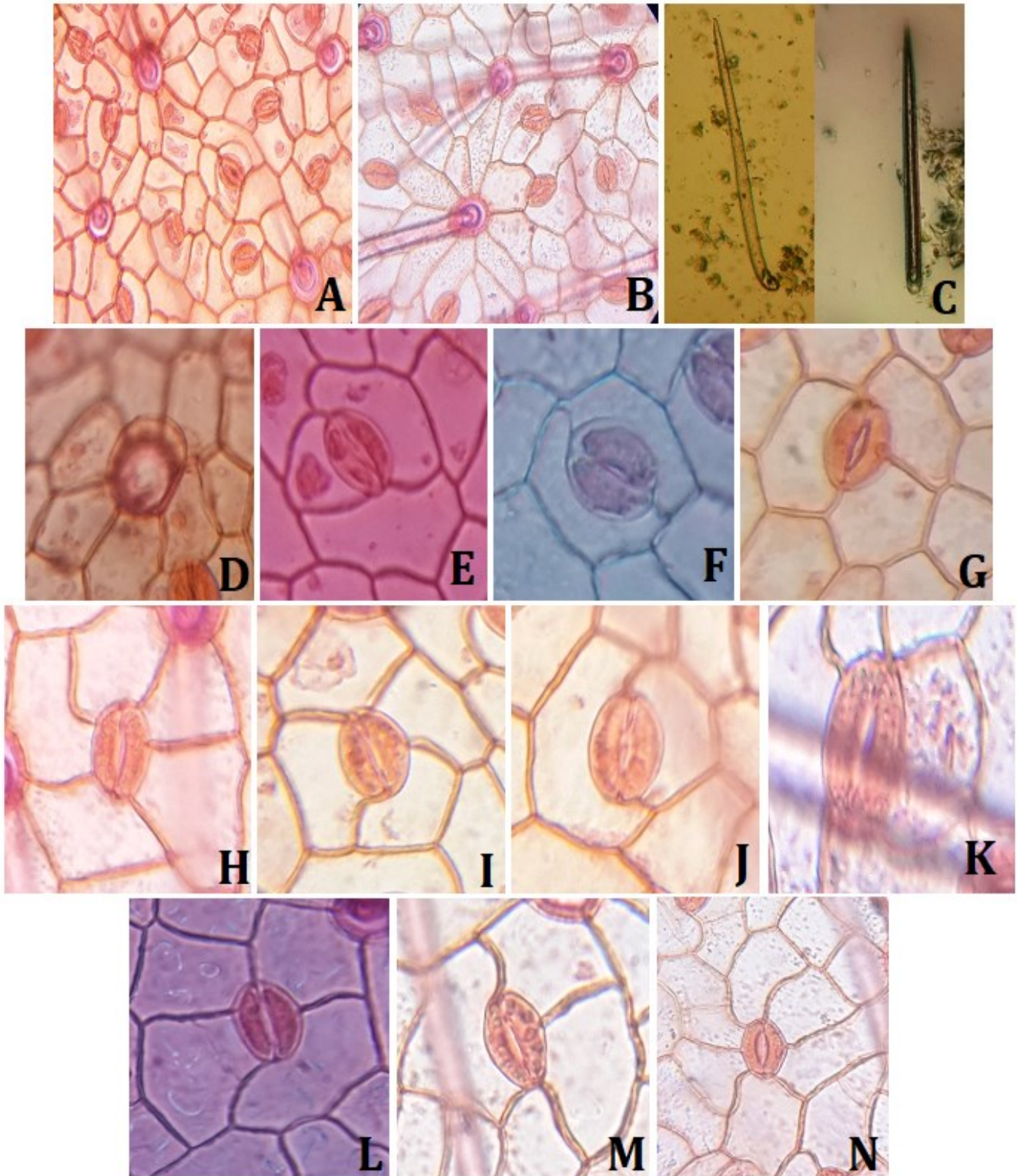
**Figure 1-** *T.purpurea* : (A) adaxial epidermis (B) abaxial epidermis(C) E glandular trichome with oblique wall and without oblique wall(D) Peltate glandular trichome (E)Anisocytic stomata (F)Paracytic stomata (G)Isotricytic stomata(H)Brachyparacytic stomata (I) Brachyparatetracytic stomata(J) Laterocytic stomata (K) Hemi paracytic stomata (L)Staurocytic stomata (M)Anomotetracytic stomata (N) Anomocytic stomata



**Figure 2 :** *T.uniflora* sub sp. *petrosa* : (A) adaxial epidermis (B) abaxial epidermis(C) E glandular trichome with oblique wall and without oblique wall(D) Peltate glandular trichome (E)Anisocytic stomata (F)Paracytic stomata (G)Isotricytic stomata(H)Brachyparacytic stomata (I) Brachyparatetracytic stomata(J) Laterocytic stomata (K) Hemi paracytic stomata (L)Staurocytic stomata (M)Anomotetracytic stomata (N) Anomocytic stomata



**Figure 3 - *T. villosa*:** (A) adaxial epidermis, (B) abaxial epidermis, (C) E glandular trichome with oblique wall and without oblique wall, (D) Peltate glandular trichome, (E) Anisocytic stomata, (F) Paracytic stomata, (G) Isotricytic stomata, (H) Brachyparacytic stomata, (I) Brachyparatetracytic stomata, (J) Laterocytic stomata, (K) Hemi paracytic stomata, (L) Staurocytic stomata, (M) Anomotetracytic stomata, (N) Anomocytic stomata



**Figure 4** - *T. wallichii* : (A) adaxial epidermis, (B) abaxial epidermis, (C) Eglanular trichome with oblique wall and without oblique wall, (D) Peltate glandular trichome, (E) Anisocytic stomata, (F) Paracytic stomata, (G) Isotricytic stomata, (H) Brachyparacytic stomata, (I) Brachyparatetracytic stomata, (J) Laterocytic stomata, (K) Hemi paracytic stomata, (L) Staurocytic stomata, (M) Anomotetracytic stomata, (N) Anomocytic stomata



Peltate glands were reported on leaf surface of all species. In *T. villosa* and *T. wallichii* they occurred on surface of leaf, present among eglandular trichomes, their density was more near vein let and leaf margins. In *T. purpurea* and *T. uniflora* sub sp. *petrosa* these glandular trichomes were present only on lower epidermis, mainly confined near to leaf margins with relatively very low frequency as compared to other two species. The size of peltate gland were largest ( $26.0 \pm 0.82 \times 24.44 \pm 1.04 \mu\text{m}$ ) in *T. wallichii* and smallest ( $20.28 \pm 0.97 \times 19.76 \pm 0.64 \mu\text{m}$ ) in *T. villosa*.

## DISCUSSION

Taxonomic significance of micro morphological characters in taxa delimitation has been described by various workers such as Dehgan (1980), Stace (1989), Ogundipe *et al.*, (2009), Albert and Sharma (2013), Chukwuma and Ayodele (2016). Distribution patterns, size and types of stomata always found to be specific for a genus and species. Earlier a few studies on micro morphological characters of various species of *Tephrosia* were carried out in India by Shah and Kothari (1976), Bhatt and Tuteja (1984), Rao and Rao (1992), but as far as concern about Indian Thar desert no serious attempt have been made on micro morphology of *Tephrosia*, therefore the present study was undertaken with the aim to provide an identification criteria of species on basis of foliar micro morphological characters.

Leaves in investigated species of *Tephrosia* were amphistomatic. Some stomatal types such as anomocytic, anisocytic, paracytic and brachyparacytic were earlier reported in *Tephrosia* by Solereder (1908), Metcalfe and Chalk (1952), Shah and Gopal (1969), Rao and Rao (1994). Present study was also confirmed the above investigations with additional finding of staurocytic, laterocytic, brachyparatetracytic, anomotetracytic, isotricytic and hemiparacytic as well. Epidermal cells were polygonal, rectangular and isodiametric in shape with straight and curved anticlinal wall pattern, which in line with the previous finding (Stace, 1965) that the plant grows in dry area tend to have straight to curved anticlinal wall.

Trichomes characters were found to be significant on genus level; they are generally constant in species group. *Tephrosia* species showed uniseriate, eglandular and peltate glandular trichomes. Eglandular trichomes were formerly referred as 'ordinary simple

papilionaceous hair' (Solereder, 1908) or '3-celled hair' (Metcalfe & Chalk, 1952). It consists of two parts, the bulbous foot and body, which was further differentiated into stalk and a head (Ramayya 1981). The wall of trichomes were thick and warty. Present study made same observations for trichomes but there was a remarkable difference in the occurrence of trichomes on both surfaces of leaf among investigated taxa. Out of the four species two species viz. *T. villosa* and *T. wallichii* were amphitrichomic, etrichomes found on both surfaces where as in other two species viz. *T. purpurea* and *T. uniflora* sub sp. *petrosa* trichomes absent on upper epidermis. Further, variations in trichomes density on upper epidermis in former two species also reported, together with variations in length of trichomes on both surfaces of leaf. Trichomes found on adaxial epidermis were short in length, whereas on abaxial side they were short as well as long type. Present study revealed that a number of trichomes have an oblique partition wall separating basal cell from the long emergent cell; where as in other trichomes basal cell and long pointed cell was without any oblique partition wall in all four species. Present study agreed with observations of other researchers reported same type of trichomes in for *T. villosa* (Chauhan and Daniel, 2011).

Presence of Peltate glands were earlier studied by Choi and Kim (2013), Essiett and Iwok (2014), Bano and Deora (2017). Peltate glands were disk shaped and made up of a single cell base, a stalk and rounded head as reported by Combrinck *et al.*, (2007) in *Lippia scaberrima*. Previously no peltate glandular trichomes were reported in *Tephrosia*.

### Taxonomic key

- 1a. Eglandular and peltate glandular trichomes present on lower surface of leaf.....2
- 1b. Eglandular and peltate glandular trichomes present on both surface of leaf.....3
- 2a. Stomatal density 9-10 on upper epidermis and 8-10 on lower epidermis .....*T. purpurea*
- 2b. Stomatal density 15-18 on upper epidermis and 11-13 on lower epidermis.....*T. uniflora* subsp. *petrosa*
- 3a. Stomatal index 17.03 on lower epidermis ..... *T. villosa*
- 3b. Stomatal index 15.60 on lower epidermis .....*T. wallichii*

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