

## Pharmacognostic Study of *Tanacetum baltistanicum* Podlech. An Endemic Species from Northern Pakistan

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### Abstract

In the present study characterization of morpho-anatomical characteristics of the leaf, stem and root of *Tanacetum baltistanicum* was carried out. Macroscopic characters of the *T. baltistanicum* revealed the presence of pilose, glabrous and green stem, green, pubescent, spirally arranged and persistent leaves, both stem and leaves possessed strong pungent aroma and bitter acrid taste. The roots were brown in colour slightly curved, aromatic and bitter in taste. Microscopic characters showed that T.S of the stem of *T. baltistanicum* possessed single layer of epidermal cells with distinct cortical parenchyma cells and vascular bundles. T.S of root has multi-layered hypodermis. T.S of leaf showed the presence of white hair on both sides of epidermis and mesophyll tissues were differentiated into spongy and palisade parenchyma. Foliar anatomy showed that stomata are amphistomatic and anomocytic. Powder drug analysis of stem, root and leaf of *T. baltistanicum* showed presence of sclerenchyma cells, epidermal cells, thick walled fibers, cork cells, trichomes and tracheids and vessels. To our knowledge, these morphological features are reported for the first time for this species and are of great taxonomic significance in identifying and authentication of plants based on morpho-anatomical characters. This study will enable the research community to develop better morphological and pharmacological standards for formulation of genuine drugs.

**Keywords:** Anatomy, Morphology, Powder Drug, Foliar Anatomy, Pharmacognosy.

## 1. Introduction

Medicinal plants are of great importance with their diverse application in the folk traditional medicine system. In the developing countries about 3.5 billion people rely on the medicinal plants for basic health care [1]. Pakistan has a great floristic diversity, there are about 6000 species of medicinal plants have been reported in Pakistan but, only 600 species have been explored from medicinal and pharmacological point of view [2]. Genus *Tanacetum* L. comprises highly medicinal, aromatic and herbaceous species. Various species of *Tanacetum* are widely being used as an antihistaminic, anti-inflammatory, and insecticidal agents [3,4]. *Tanacetum parthenium* L. (Feverfew) is traditionally used for treatment headache, migraine, fever, arthritis and stomach pain, Hay fever, asthma, dizziness, nausea, and vomiting [5]. *Tanacetum baltistanicum* Podlech. is an endemic plant species belonging to the family astraceae commonly confined to the dry, sandy and rocky patches of the Gilgit-Baltistan (Northern Pakistan). Plant is 15-30cm tall, stems pilos, leaves 1-2cm are smooth and petiolate (Figure-1). *T. baltistanicum* is traditionally used for treatment of wounds by local community. Decoction of leaves has got inflammatory properties. Due to its strong aroma leaf smoke is used as mosquito repellent and used as insecticidal agent. To best of our knowledge this is the first systematic study on the morpho-anatomical characteristics of *T. baltistanicum*. No previous work has been reported on this species.



Figure-1. *Tanacetum baltistanicum* Podlech.

## 2. Materials and Methods

### 2.1 Collection and authentication

The fresh samples of the *T. baltistanicum* were collected from Gilgit Baltistan (Village Karimabad), Pakistan. The collected specimens were identified, dried, pressed and mounted on standard herbarium sheets. The voucher specimens were deposited at the Herbarium of the Department of Botany, Hazara University Mansehra (Voucher Specimen No: HUP 9082). Plant specimens were thoroughly washed, and stem, root and leaves were separately dried and fine powder was made by electrical grinder. Macroscopic and morphological examination and organoleptic evaluation of stem, root and leaves was done by following the standard method of [6,7]

### 2.2 Anatomical study

The anatomical studies of leaf, stem and root of *T. baltistanicum* was accomplished by free hand section cutting. T.S of leaf, stem and root was done by sharp razor. The fine and thin section of each part was stained with safranin for 3-5 min and dehydrated with different grades of alcohol (10, 30, 50, 70, 90 and 100%). Afterwards a drop of light green was added to the sections and then dehydrated with absolute alcohol for 2-3 min. Finally, the sections were mounted in Canada balsam to make permanent slides. The slides were examined under microscope and images were captured by Camera fitted microscope (Optica-C-B5). The average size of cells and tissues of different parts was determined by ocular and stage micrometer.

### 2.3 Foliar epidermal anatomy

Foliar epidermal anatomy of *T. baltistanicum* was carried out by following the standard method of Ahmad *et al.* [8] with few modifications. Fresh leaves are cut into small fine pieces and placed into the test tubes boiled with 3mL of nitric acid and 0.2 g potassium chloride. After half an hour thin layer of epidermis was peeled off. Abaxial and adaxial surface was separated and washed with ethyl alcohol. Afterwards a drop of lactic acid is added to clean the slide and subjected to microscopic examination.

## 2.4 Powder Drug Analysis

Powder drug study was done by light microscopy (LM) in Pharmacognosy Lab, Department of Botany, Hazara University, Mansehra, Pakistan. Finally dried powder of all the parts was through sieve (fine sieve no.60) and then boiled in the 70% concentrated chloral hydrate solution for 10-15 min, macerated in iodine solution and glycerin on a glass slide and subjected to microscopic examination and photography by camera fitted microscope (Optica-C-B5).

## 3. Results and Discussion

### 3.1 Morphological observations of leaf, stem, and root

*T. baltistanicum* is an endemic plant found in Gilgit Baltistan region in dry, sandy, and rock hills, flowering period from August to September. *T. baltistanicum* has spirally arranged, green, persistent leaves, herbaceous and pubescent with bitter acrid taste and strong aroma. Lamina simple and ovate. Leaf base attenuate, apex trifid and unicostate, venation is reticulate. Stem is pilose, green, glabrous, strongly aromatic, and bitter in taste. Fracture is short and fracture with smooth surface. Root cylindrical, slightly curved with rootlets, brown in colour, strongly aromatic with bitter acrid taste. Macroscopic features of the leaf, stem, and root of *T. baltistanicum* that is shape, odour, colour and taste (organoleptic features) are listed in (Table 1-3). Bercu *et al.* [9] reported the similar results for the morphological features of leaf, stem, and roots of *Astertripolium* belonging to the family Asteraceae which are in consistency with our results of *T. baltistanicum*.

**Table-1. Macroscopic Features of Leaf of *T. baltistanicum***

S. No	Features	<i>Tanacetum baltistanicum</i>
1	Size	Length 1 – 2 cm, Width 0.3-0.8 Cm
2	Duration	Deciduous
3	Color	fresh green colour
4	Taste	Bitter, unpleasant
5	Odour	Strongly aromatic
6	Phyllotxis	Spiral alternate
7	Insertion	Ramel and Cauline
8	Leaf base	Attenuate
9	Petiole, stipule	Sessile
10	Lamina	Simple and Ovate
11	Incision	Margin serrate, dentate,
12	Venation	Reticulate
13	Apex	Trifid
14	Surface	Without hair
15	Texture	Herbaceous
16	Fracture	Short and smooth

**Table-2 Macroscopic Features of stem of *T. baltistanicum***

<b>S.N O</b>	<b>Features</b>	<b><i>Tanacetum baltistanicum</i></b>
1	Colour	Dark Green
2	Odour	Strongly aromatic
3	Shape	Pilose, Glabrous
4	Taste	Acrid and bitter
5	Thickness	0.5 cm -2 cm
6	Fracture	Short
7	Surface of Fracture	Smooth
8	Texture	Herbaceous, older woody

**Table-3 Macroscopic Features of root of *T. baltistanicum***

<b>S.NO</b>	<b>Features</b>	<b><i>Tanacetum baltistanicum</i></b>
1	Colour	Brown
2	Odour	Strong aromatic
3	Shape	Cylindrical, slightly curved
4	Taste	Sour
5	Rootlets	Present
6	Root Form	Tape root
7	Fracture	Short
8	Fracture	Hard
9	Texture	Fibrous

### 3.2 Anatomical Features

#### 3.2.1 Root

Transverse section of the root showed the cortex surrounded by a multicellular hypodermis layer. Cortex parenchymatous, 13-15 layered thick which is followed by a layer of endodermis containing stele in the center. Stele contained pericycle with conducting tissues i-e xylem and phloem. Sclerenchymatous cells and medullary rays were present between xylem tissues. Xylem vessels large and radially arranged containing xylem fibers. Phloem tissue contained phloem vessels. Pith large with metaxylem elements. Cambium more distinct in between xylem and phloem tissue. (Figure-2)

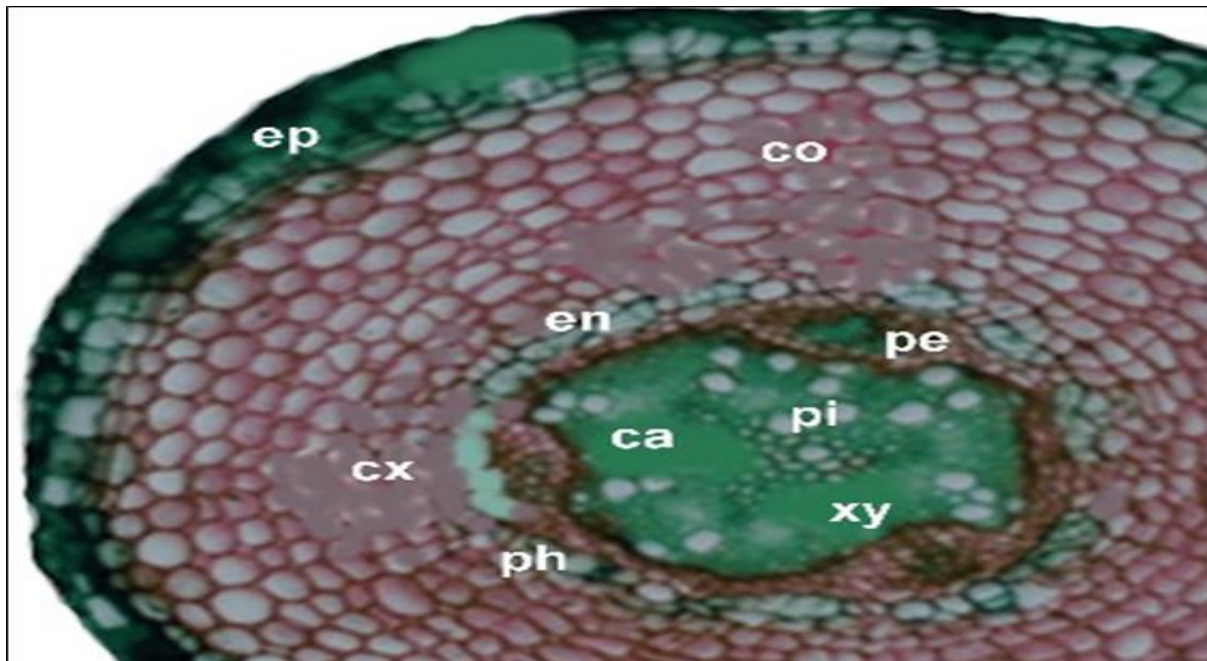


Figure-2 T.S of Root of *T. baltistanicum*, ep: eipdermis, co: cortex, en: endodermis, pt: pith, cr: cork, xy: xylem, ca: cambium, ph:phloem,

#### 3.2.2 Stem

Stem possessed the outer most layer of epidermis with single layered uniseriate cells that may be oval or squarish in shape, covered by a layer of cuticle. Below the epidermis there was 7–8-layer cortex the cortex with large number of parenchyma cells and the secretory ducts over endodermis. The endodermis being very prominent, and it is the inner layer of the cortex. Vascular bundles collateral type 14-17 in number and surrounded by sclerenchyma fibers. Vascular cambium

contained xylem and phloem but not well differentiated. Medullary rays present in the form of concentric ring. Pith large, thick and parenchymatous (Figure-3). Similar studies for anatomical features of six endemic species of *Tanacetum* from Turkey have been reported by Tekin *et al.* [10].

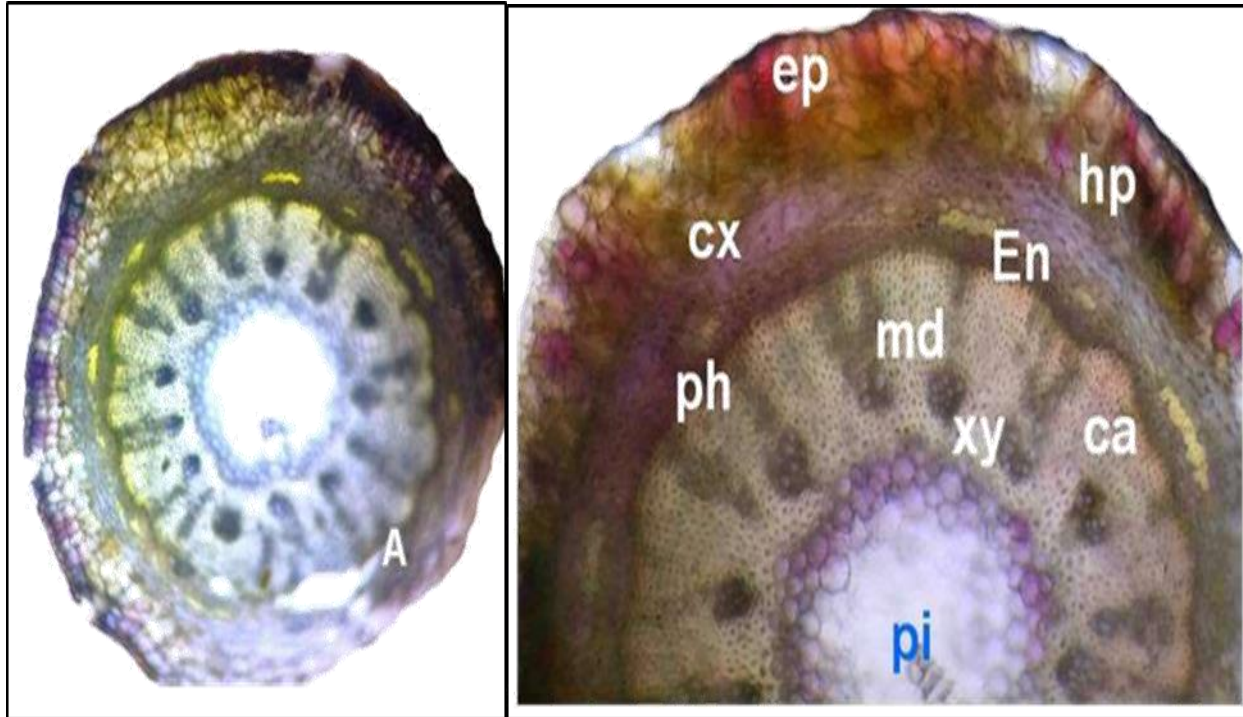


Figure-3 T.S of Stem of *T. baltistanicum*, ep: epidermis, cr: cortex, en: endodermis, pt: pith, xy: xylem, ca: cambium, md, medullary rays, ph:phloem, hp: hypodermis

### 3.2.3 Leaf

Simple white hairs were present on both the adaxial and abaxial surface of leaf. The stomata anomocytic that is the stomata were present on both sides of epidermis (upper and lower surface), the cells of stomata were located on the similar position with the epidermal cells. Palisade and spongy parenchyma can easily be distinguished present at both sides of the leaf, the palisade parenchyma cells were double layered, while 2-3 layered thick spongy parenchyma can be seen in the area between upper and lower layer of palisade parenchymatous cells. Collateral type of vascular bundles surrounded by a parenchymatous bundle sheath. Xylem and phloem cannot be differentiated in cambium. (Figure-4)





Figure-4. T.S of Leaf of *T. baltistanicum* up: upper epidermis, pp: palisade parenchyma, sp: spongy parenchyma, xy: xylem, ph:phloem, le: lower epidermis

### 3.3 Foliar Epidermal Anatomy

Stomatal apparatus of *T. baltistanicum* appeared to be anomocytic and stomata were present equally on both abaxial and adaxial surfaces. *baltistanicum* stomatal number and stomatal index values were in the range of 120 to 140 (130) and 10 to 13 (11.5) per mm<sup>2</sup>. (Table-4, Figure-5). Leaf constant parameter have also been reported by various other workers. Tekin *et al.* [10] proposed the foliar anatomical features of six endemic species of *Tanacetum* from Turkey. Barkatullah [11] reported the similar foliar anatomical characters of *S. laureola* and *Z. armatum* of family rutaceae. Their results were more or less similar to our findings.

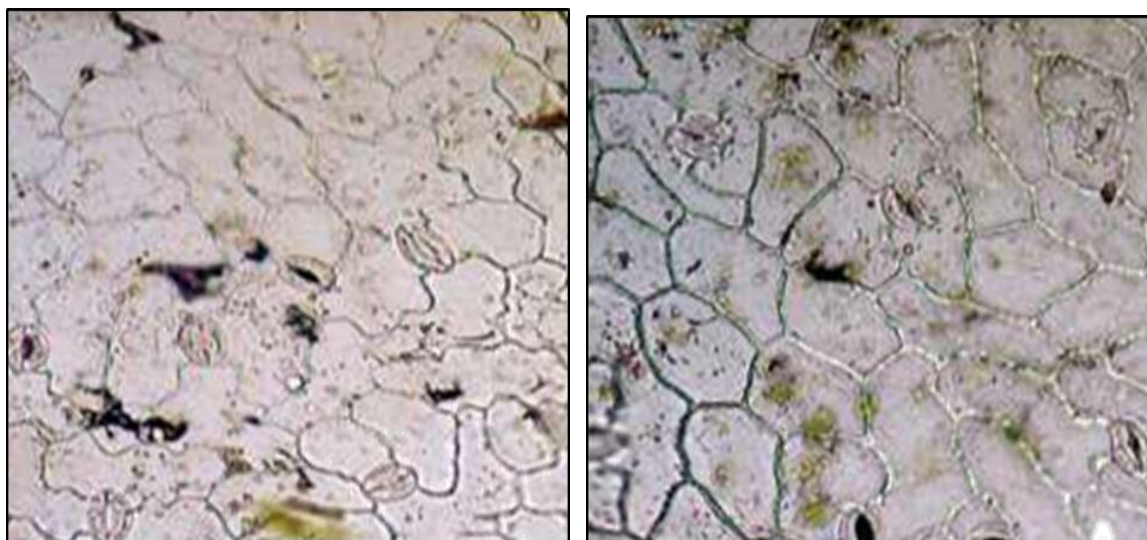


Figure-5 A: Adaxial surface of Leaf

B: Abaxial surface of Leaf

Table-4 Leaf Constant Parameters of *T. baltistanicum*

Character	Range	Average
PR	6-8	7
VIN	10-14	12
VTN	8-10	9
SN	120-140	130
SI	10-13	11.5

PR= Palisade Ratio, VIN= Vein Islet Number, VTI= Vein Termination Number, SI SN = Stomatal Number, = Stomatal Index.

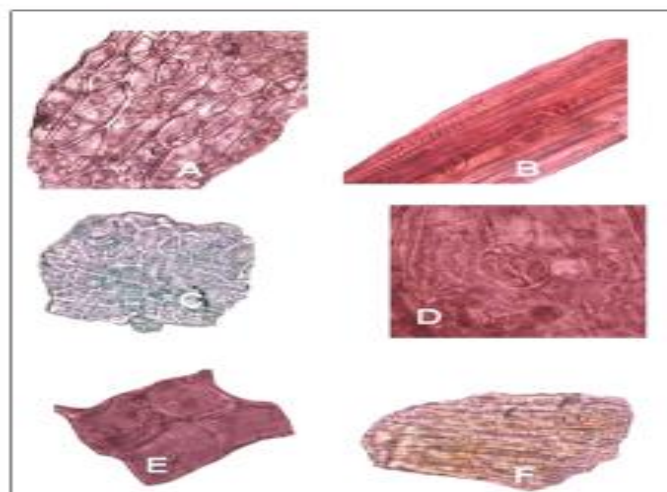
### 3.4 Powdered drug analysis of different parts *T. baltistanicum*

#### 3.4.1 Leaf powder

Leaf powder of *T. baltistanicum* was dark green in colour, with pungent odour and bitter unpleasant taste. Microscopic analysis of the powder of leaf possessed elongated cork cells with oil droplets, different types of tracheids and fibers with vessels of reticulate and pitted thickening, fragments of upper and lower epidermis showing stomata, fragments of cortical tissue and and parenchyma cells with crystals of calcium oxalate. Quantitative parameters i-e length and width of powdered drug of *T. baltistanicum* leaf are explained in (Table- 5, Figure-6)

**Table-5. Microscopic Features of powder drug of leaf of *T. baltistanicum***

S.No	Cell Type	Length ( $\mu\text{m}$ )	Width ( $\mu\text{m}$ )
1	Epidermal cell	28	16
2	Parenchyma cell	44	13
3	Sclerenchyma cell	26	8
4	Trichome	74	7
5	Pitted tracheid	132	18
6	Spiral tracheid	68	22
7	Reticulate tracheid	84	22
8	Cork Cells	36	18



**Figure-6 Powder Microscopy of *T. baltistanicum* Leaf, A: Cork cells, B: Tracheids with fibers & fessels, C: Epidermal cells, D: Epidermal cell with stomata, E: Cortical Parenchyma Cells, Parenchyma cells with calcium oxylate**

### 3.4.2 Stem powder

Powdered drug of the *T. baltistanicum* stem appeared bold green with strong pungent odour & bitter acrid taste. Microscopic studies showed the presence of thick-walled elongated sclerenchyma cells, epidermal cells, thick walled fibers, trichomes and tracheids with different types of vessels. Quantitative parameters i-e length and width of powdered drug of root of *T. baltistanicum* are explained in (Table-6, Figure-7)

**Table-6** Microscopic Features of powder drug of stem of *T. baltistanicum*

S.No	Cell Type	Length ( $\mu\text{m}$ )	Width ( $\mu\text{m}$ )
1	Epidermal cell	26	18
2	Parenchyma cell	42	10
3	Sclerenchyma cell	24	16
4	Trichome	82	8
5	Pitted tracheid	144	18
6	Spiral tracheid	76	32
7	Reticulate tracheid	106	24

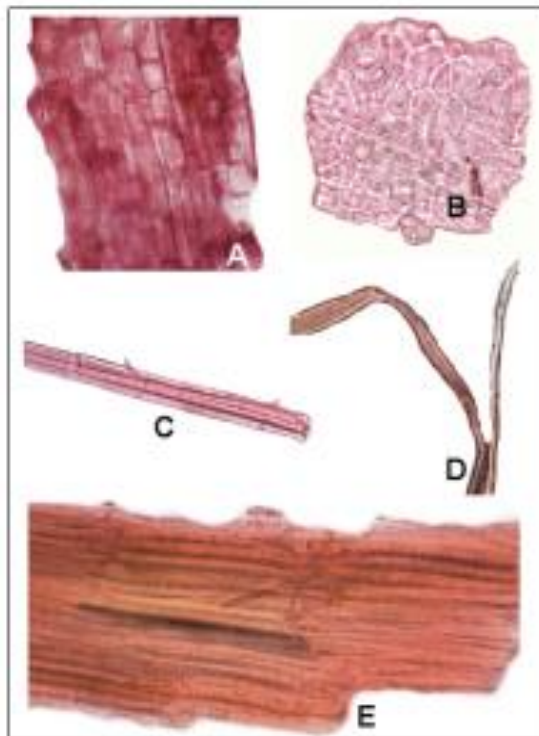


Figure-7 A; Powder Microscopy of *T. baltistanicum* Stem, A: Sclerenchyma cells, B: Epidermal cells, C: Thick walled Fibers, D Trichomes, E: Tracheids and vessels.

### 3.4.3 Root powder

Powder drug of the root of *T. baltistanicum* appeared light brown in colour, odour less aromatic and slight bitter taste. The powder drug study of the root showed epidermal cells with parenchyma, cork cells with oil droplets, sclerenchyma cells, tracheids with multi fibers, vessels with anular and spiral thickening and thick-walled fibers. Quantitative parameters i-e length and width of powdered drug of root of *T. baltistanicum* are explained in (Table- 7, Figure-8).

**Table-7. Microscopic Features of powder drug of root *T. baltistanicum***

<b>S.No</b>	<b>Cell Type</b>	<b>Length (<math>\mu\text{m}</math>)</b>	<b>Width (<math>\mu\text{m}</math>)</b>
1	Epidermal cell	68	12
2	Parenchyma cell	58	8
3	Cork cells	72	6
4	Sclerenchyma cells	114	10
5	Pitted tracheid	116	32
6	Spiral tracheid	64	24
7	Scalariform tracheid	78	11
8	Thick walled fiber	62	14
9	Vessels	68	22

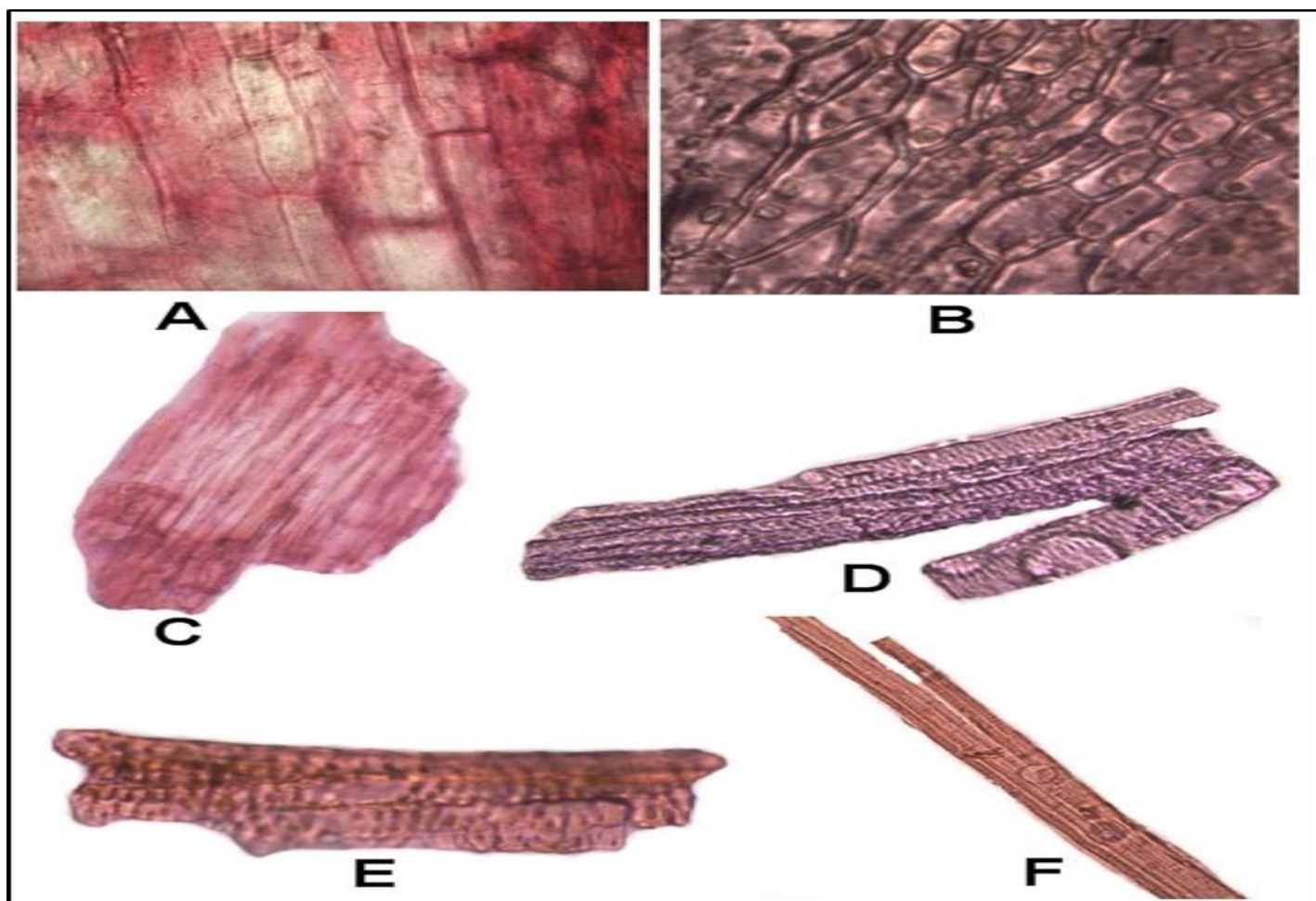


Figure-8 Powder Microscopy of *T. baltistanicum* Root,

**A: Epidermal Parenchyma, B: Cork cells, C: Sclerenchyma Fibers, D: Tracheids with Multifibers, E: Vessels, F: Thick walled fibers**

Powder microscopy of different plant species have been reported by various researchers. Histochemical studies of the various plants of family Solanaceae was carried out for authentication and classification of medicinal plants [12]. Powder drug analysis of *Tridax procumbens* revealed the similar histological characters [13]. Powder microscopy of *Ficus bengalensis* showed the presence of epidermal cells, fibers, trichomes and calcium oxylate crystals of [14]. Histological characters of *Calotropis procera* were studied by Murti *et al.* [15]. Power drug analysis of *Sesbania sesban* revealed the presence of xylem vessels, pitted xylem fibers, xylem parenchyma, calcium oxylate crystals and starch grains and multiseriate medullary rays [16]. Histology of root of *Chenopodium album* determined the presence of starch grains, different type of vessels, fibers and parenchyma cells [17]. Root of *Atropa belladonna* root revealed distinct parenchyma cells,

medullary rays and starch grains [18]. Powder drug study of *Datura stramonium* identified definite number of stomata, non glandular trichomes, crystals of calcium oxalate palisade parenchyma and spongy parenchyma [19].

### Conclusion

The results of this study revealed that the morpho-anatomical features possessed huge diversity and have potentially improved the taxonomic identification of this species that could be exploited as important taxonomic marker. Pharmacological parameters could be a significant tool for discovery of innovative drugs.

### Conflict of Interest

Authors declare no conflict of interest

### References

- [1] Idu, M., & Onyibe, H.. Medicinal plants of Edo state, Nigeria. *Research Journal of Medicinal Plant*, (2007),1(2), 32–41.
- [2] Shinwari, Z. K. Medicinal plants research in Pakistan. *Journal of medicinal plants research*, (2010), 4(3), 161–176.
- [3] Baser, K.H.C., Demirci, B., Tabanca, N., Ozek, T., & Goren, N. Composition of the essential oils of *Tanacetum armenum* (DC.) Schultz Bip., *T. balsamita* L., *T. chiliophyllum*(Fisch.& Mey.) Schultz Bip. var. *chiliophyllum* and *T. haradjani* (Rech. fil.) Grierson and the enantiomeric distribution of camphor and carvone, *Flavour Frag J.*, (2001). 16: 195–200.
- [4] Williams, C.A., Harborne, J.B., Geiger, H., Hoult, J.R. The flavonoids of *Tanacetum parthenium* and *T. vulgare* and their anti-inflammatory properties,*Phytochemistry*,(1999), 51:417–23.
- [5] Pareek, A., Suthar, M., Rathore, G. S., & Bansal, V. Feverfew (*Tanacetum parthenium* L.): A systematic review. *Pharmacognosy reviews*, (2011), 5(9), 103.
- [6] Evans, W. C. Trease and Evans, pharmacognosy London, UK: Elsevier Health Sciences Publishers, (2009), (16th ed., pp. 553–557).
- [7] Wallis, T. E. Text book of pharmacognosy. New Delhi, India: CBS Publisher and Distributors, (2009),(5th ed., pp. 572–575).



- [8] Ahmad, M., Khan, M. A., Rashid, U., Zafar, M., Arshad, M., & Sultana, S. Quality assurance of herbal drug valerian by chemotaxonomic markers. *African Journal of Biotechnology*, (2009), 8(6), 1148–1145.
- [9] Bercu, R., Fagaras, M.L., & Broasca, L. Anatomical features of *Aster tripolium*(Asteraceae) to saline environments. *Annals of RSCB.*, (2012), *Xvii (1)*, 271-277.
- [10] Tekin, M., & Kartal, C. Comparative anatomical investigations on six endemic *Tanacetum* (Asteraceae) taxa from Turkey. *Pakistan Journal of Botany*, (2016), 48(4), 1501-1515.
- [11] Barkatullah. Pharmacognosy of *Skimmia laureola* (Dc.) Siebold. & Zucc. ex Walp. and *Zanthoxylum armatum* DC. Family *Rutaceae*. (2012), Ph. D. Thesis. Submitted to Department of Botany, University of Peshawar.
- [12] Maiti, R. K., Villarreal, A., Trevino, A., & Valades-cerda, M. C. Some aspects on Pharmacognosy of ten species of the family Solanaceae utilized in traditional medicine. *Caldasia*, (2002), 24 (2): 317-321.
- [13] Mishra, S. B., Sangameswaran, B., Prajapati, K., Singh, D., & Dubey, P. Pharmacognostical and Preliminary Phytochemical studies of leaves of *Tridax procumbens* L. *Ethnobotanical Leaflets*, (2008), 12 (15): 1283-89.
- [14] Patil, V. V., Pimprikar, R. B. & Patil, V. R. Pharmacognostical studies and evaluation of anti-inflammatory activity of *Ficus bengalensis* Linn. *Journal of Young Pharmacists.*, (2009), 1(1): 49-53.
- [15] Murti, Y., Yogi, B., & Pathak, D. Pharmacognostic standardization of leaves of *Calotropis procera* (Ait.) R. Br. (Asclepiadaceae). *Int. J. Ayurveda Res.*, (2010), 1 (1): 14–17.
- [16] Nirmal, S. A., Bairagi, J. H., Zaware, B. B., Dighe, N. S. & Digh, S. B. Pharmacognostic study of *Sesbania sesban* wood. *Inter. J. Pharma Res. & Develop*, (2009), 1 (9): 1-4.
- [17] Yadav, N., Vasudeva, N., Sharma, S. K., & Singh, S. Pharmacognostic and phytochemical studies on *Chenopodium album* Linn., root. *Hamdard Medicus*, (2007), 50(1): 95 – 102.
- [18] Youngken, H. W. A Text Book of Pharmacognosy. *The Blackiston Co Toronto, Philadelphia*, (1950), pp. 1675 – 1676.

[19] Evans, W. C. *Trease and Evans Pharmacognosy*. W. B. Saunders Publisher, London, New York, (2009), 16<sup>th</sup> ed. pp. 480.

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