



PHARMACOGNOSTIC EVALUATION OF LEAF, STEM AND ROOT OF *TALINUM CUNEIFOLIUM* LINN

Yasodha Krishna Janapati^a, Sunil Junapudi^b, Were L. L. Munyendo^a

^aSchool of Pharmacy & Health Sciences, United States International University – Africa
P.O. Box 14634 – 00800, Nairobi, Kenya.

^bDepartment of Pharmaceutical Chemistry, Geethanjali College of Pharmacy, Cheeryal,
Keesara, Medchal District, Telangana, India- 501301.

*Corresponding author E-mail: krishna.yasodha@gmail.com

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ABSTRACT

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A wide range of pharmacological activities have been reported for the erect shrub with subterranean tuber, *Talinum cuneifolium*. The presents paper of a Pharmacognostic evaluation of leaf, stem and root of *Talinum cuneifolium* Linn (portulaceae); the investigations focused at macroscopic, microscopic characters of leaf, stem, root and powder. Microscopy of *Talinum cuneifolium* leaf showed the presence of midrib, mucilage filled idioblast, xylem, phloem, paracytic stomata and guard cells. Wide cortex, periderm, starch grains and calcium oxalate crystals were present in microscopy of root. Powder microscopy showed the presence of calcium oxalate druses and small fragments of lamina. This forms a fundamental basis for developing histological standards of raw materials and its characterization of medicinal botanicals.

INTRODUCTION

Over the last decade era, there has been a emergent interest in drugs of herbal origin in divergence to the synthetics that are viewed as unsafe to human and environment¹. Now days, Herbal medicines are manufactured on large scales where the manufacturers are facing problems such as availability of good quality raw material because of adulteration, substitution (intentionally and unintentionally), authentication of raw material, availability of standards, proper standardization methodology of drugs and formulation i.e. quality control parameters². Many researchers indicate both the macro and microscopic characters often help in correct identification of cured drug³. *Talinum Cuneifolium* Linn. of the family Portulaceae is commonly known as Ceylon bachali in English, Palaku, Akukoora, Seema

bachali in Telugu, Pasali in Tamil. This plant is commonly found in Andhra Pradesh and TamilNadu region of India, Srilanka, Bangladesh, Pakistan, U.S.A., Puerto Rico and Virgin Islands. Leaves and roots are medicinally important parts. Powdered leaf contain been reported to exhibit several efficacies for different conditions including diabetes⁴, inflammation⁵, hepatitis, mouth ulcers and is also an aphrodisiac⁶. The fresh leaves are used as stomachic. Roots possess tonic properties, used in treatment of cough, gastritis and pulmonary tuberculosis. They are also used to treat dehydrating diarrhea. With the above uses, we found *Talinum cuneifolium* as an important medicinal botanical, so it can be easily adulterated with low grade or other species if the supply of

crude drug is inadequate. This adulteration can be prevented by various evaluation parameters like microscopic, chemical study. Microscopy and powder analysis is an important tool for authentication, assist in standardization, guarantee quality, purity, and identification of crude drug⁷.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Talinum cuneifolium* was collected from S.V. U campus, Tirumala gardens of Chittoor district, Andhra Pradesh, India, in the month of October and authenticated by Asst. Prof. Dr. k. Madhava Chetty, Dept. of Botany S.V. University, Tirupathi. A.P. Specimen vouchers of *Talinum cuneifolium* was deposited at Department of Pharmacognosy for further reference.

Pharmacognostic Studies

Macroscopic Evaluation

Various macroscopic characters of fresh leaves of *Talinum cuneifolium* were studied, type of leaf base, presence or absence of petiole and characters of lamina. Lamina consists of characteristic features such as composition, incision, shape, venation, margin, apex, base, surface and texture. The root bark is morphologically studied for its size, shape, surface, fracture and configuration⁸.

Microscopic studies

The microscopic studies of the plant were carried out according to the method of Johansen⁹ and Wallis¹⁰. Fresh leaves stem and roots were separated from the plant and thoroughly washed with running water to remove the adherent impurities. Leaves and roots were air dried, powdered and stored in air tight containers for powder analysis. Fresh collected plant material (leaf, stem and root) were used for free hand section cutting and were fixed in FAA (Formalin 5 ml + Acetic acid 5 ml + 70% Alcohol 90 ml) and dehydrated with TBA as per the schedule given by Sass¹¹. TBA dehydration series was prepared table 1. The paraffin embedded specimens were sectioned with the help of Rotary Microtome. Dewaxing of the sections was done by procedure explained by Johansen⁹.

The sections were stained with Toluidine blue, Safranin and IKI - Lugol's iodine as per the method of Brien¹². For studying the leaf constants like stomatal morphology and trichome distribution Jeffrey's maceration fluid Sass¹¹ method were prepared. Different cell components were studied and measured. Photographs of different magnifications (40x and 100x) were taken with nikon alpha photo-2 microscopic unit. Descriptive terms of the anatomical features are taken from the standard anatomy book Esau¹³.

Powder Microscopy

The powder microscopy of shade dried *Talinum cuneifolium* was carried out for identification of powder characteristics by using camera¹⁴⁻¹⁶.

RESULTS AND DISCUSSIONS

Macroscopic Evaluation

It is a moderate herb having alternate, spatulate (or) oblanceolate, obscurely nerved leaves with an inflorescence terminal compound raceme. The flowers are usually small, pink in terminal and sub-dichotomously barebed panicles. It displays 2 perianth sepals, 5 petals, a unilocular gynoecium, a superior ovary with numerous ovules. The plant yields 3 central fruits that are decurved in form of a capsule globose 3-valved fruit sets (Figure-1)¹⁷. The previous workers have described morphological characters as one of the parameter for organoleptic identification of crude drugs^{18,19}.



Figure 1.0: Exomorphic Features of the *Talinum Cuneifolium* Linn

Microscopic features of *Talinum cuneifolium* Linn

Leaf: The leaf had prominent projected midrib and uniformly thick lamina. The midrib had narrow, deep adaxial groove, semicircular or broadly conical adaxial part. The semi-circular midrib was 1.1 mm thick in vertical plane and 1.8 mm broad in horizontal plane (Figure-2). The conical midrib was 1.35 mm thick and 1.6 mm wide (Figure- 4 & 5). The midrib had distinct and thick epidermal layer of thin walled squarish cells with thin cuticle, the thickness of epidermis was 20 mm. The ground tissue of the midrib was homogenous, parenchymatous cells were wide, thin walled, angular and compact. Mucilage filled idioblasts were found frequently in the midrib ground tissue (Figure -7). Tanniferous cells were seen occasionally in cells (Figure -6). The vascular strand of the midrib was broad and expanded. In the basal part of the leaf, the vascular strand was partially cleaved into two lobes (Figure-3). The conical part of the midrib was single, flat shaped or elliptical vascular strand which consists of wide, thick walled angular xylem elements. The xylem elements were less regular in distribution (Figure-3) or more or less in radial rows (Figure-5). Phloem occurs in three or four thick segments, two or three layers of thick walled parenchyma cells were located beneath the phloem segments (Figure-3, 5, 6). The vascular strand was 150-200 μm in vertical axis and 200-600 μm in horizontal axis.

8: Abaxial epidermis with stomata; Figure 9: Adaxial epidermis with stomata.

Lamina: The lamina was amphistomatic (stomata on both upper and lower sides) with reference to the mesophyll tissue. The thickness of lamina was 250 mm. The mesophyll had four or five layers of cells of varying shape and size (no distinct palisade cells). Some of the mesophyll cells were modified into the wide mucilage filled idioblasts, such mucilaginous cells were seen in the midrib. The leaf margin was blunt and conical. The epidermal cells at the extreme end were dilated into a semicircular structure. The submarginal part had small, compact, fairly thick walled mucilage filled parenchyma cells. The marginal portion was 70 μm thick.

Epidermal cells and stomatal morphology

The epidermal cells of the adaxial side were wider and have more or less straight walls (Figure-9). The abaxial epidermal cells were comparatively narrow and their walls were undulate rendering them amoeboid outline (Figure-8). The epidermal cell walls were fairly thick and smooth. No cuticular striations were evident.

Stomata were predominantly paracytic type on both sides of the lamina. A pair of subsidiary cells, one on either side of the stoma were present. They were oblong and parallel to the guard cells (Figure-9). The guard cells were 15x20 μm in size.

Stem

Young stem: Young stem was circular with uneven outline. It exhibits primary state of growth; it had an intact epidermis, narrow cortex, central pits and a circle of about 14 discrete vascular bundles (Figure-10).

Epidermis: The epidermal layer was narrow with small, thin walled rectangular measuring 20 μm in width. Cortex was 500 μm wide, had outer zone of four or five layers of collenchyma and inner zone of about seven layers of thin walled and compact parenchyma (Figure-11). Pith was wide, homogenous and parenchymatous. Some of the pith and cortical cells were dilated into mucilage containing idioblast.

Old stem: The old stem was similar to the young stem except some difference in the vascular tissues. The vascular bundles had prominent sclerenchyma cap on the phloem side. A thin cambial zone was seen between vascular bundles which will produce the secondary xylem during later stage of growth. Phloem tissue consists of scattered sieve elements and phloem parenchyma cells. The sieve elements were narrow with small companion cells along the corners (Figure-12). The xylem elements were 200 μm in diameter (Figure-13).

Root: The root was thick, fleshy and tuberous. It was circular in cross sectional outline. It consists of a narrow superficial periderm, wide cortex and a circular solid cylinder of vascular tissues.

Table 1: TBA dehydration series was prepared as follows

Sl. No	Distilled water (ml)	TBA (ml)	Ethyl alcohol 95% (ml)	Ethyl alcohol 100% (ml)	Time in hours
1	50	10	40	-	2
2	30	20	50	-	12
3	15	35	50	-	1
4	-	55	45	-	1
5	-	75	-	25	1
6	-	100	-	-	1
7	-	100	-	-	12
8	-	100	-	-	1

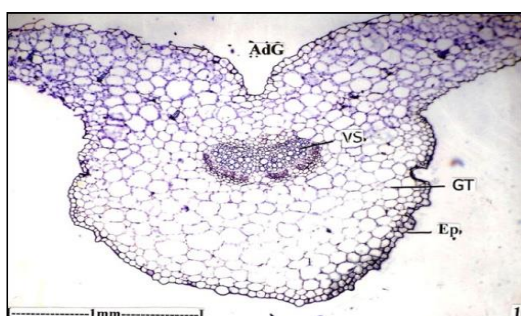


Figure: 2

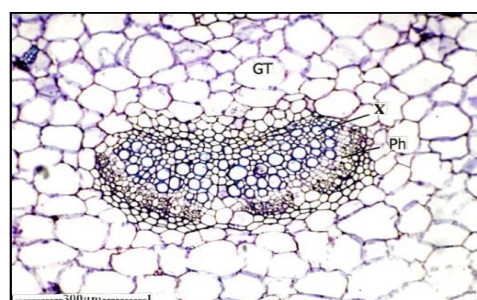


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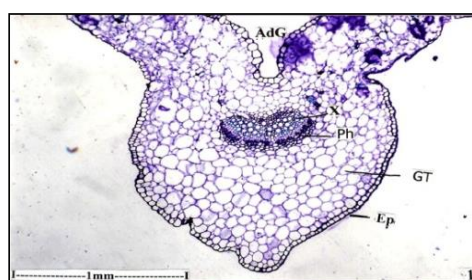


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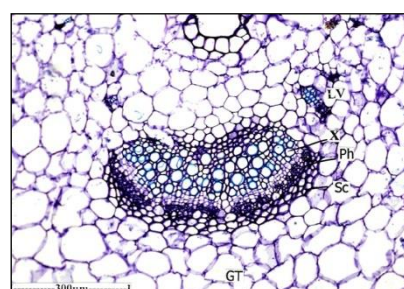


Figure: 5

ADG- Adaxial Groove; EP- Epidermis; X- Xylem; GT- Ground Tissue; PH- Phloem; VS- Vascular Strand; LV- Lateral View; PH- Phloem; SC- Sclerenchyma

Figure 2: T. S of leaf through basal part of midrib with lamina; Figure 3: Midrib vascular bundle enlarged; Figure 4: T.S of leaf through middle part of the midrib. Figure 5: Midrib vascular bundle enlarged.



Figure 6

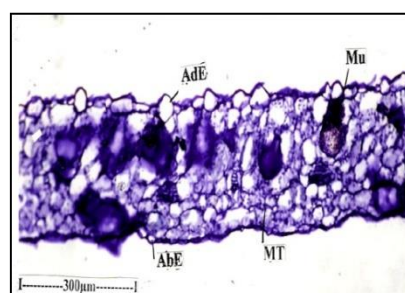


Figure 7

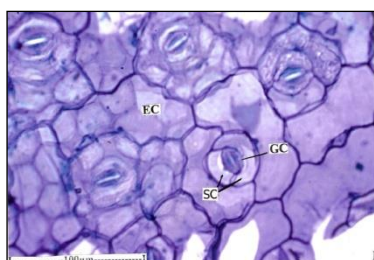


Figure 8

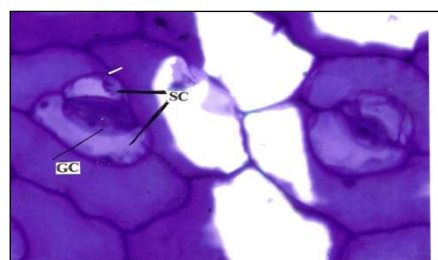


Figure 9

SC- Sclerenchyma; ADE- Adaxial Epidermis; ABE- Abaxial Epidermis; MT- Mesophyll Tissue; MU- Mucilage Cells; TA- Tanniferous Cells; EC- Epidermal cell; GC- Guard Cell; SC- Subsidiary Cells

Figure 6: T. S of leaf through terminal part of the midrib; Figure 7: T. S of lamina; Figure

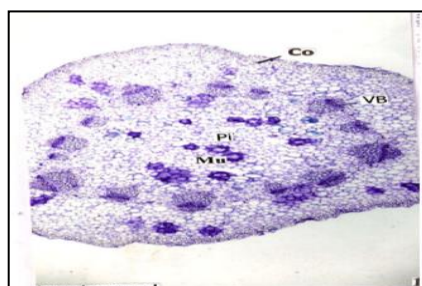


Figure 10

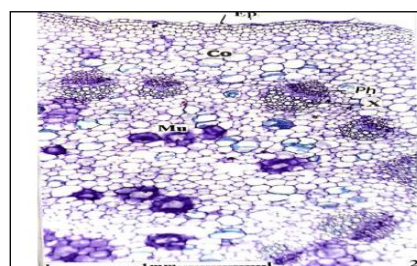


Figure 11

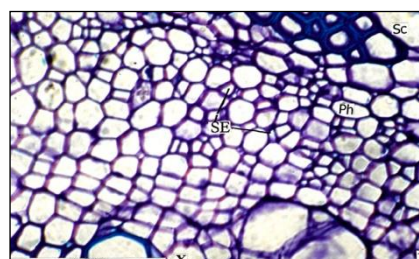


Figure 12

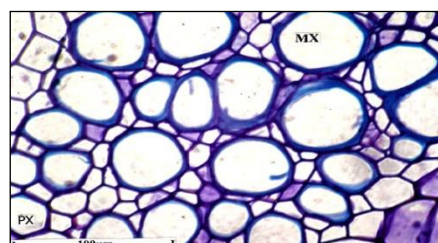


Figure 13

CO- Cortex; EP- Epidermis; MU- Mucilage Idioblast; PH- Phloem; PI- Pith; VB- Vascular Bundle; MX- Meta Xylem; PX- Proto Xylem; SC- Sclerenchyma Cells; SE- Sieve Element; X- Xylem

Figure 10: T. S of stem entire view; Figure 11: T. S of stem a sector enlarged; Figure 12: T. S of phloem showing sieve-elements; Figure 13: T. S of stem showing proto xylem and meta xylem.

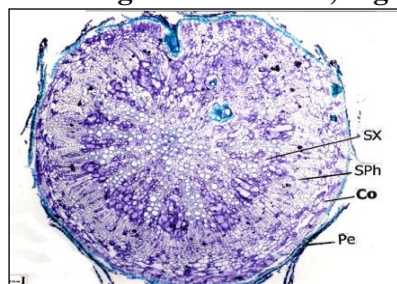


Figure 14

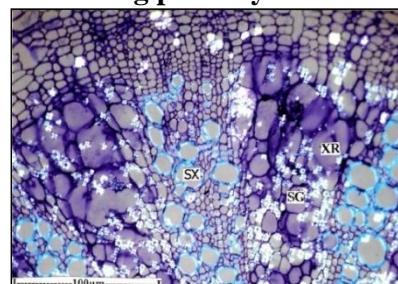


Figure 15

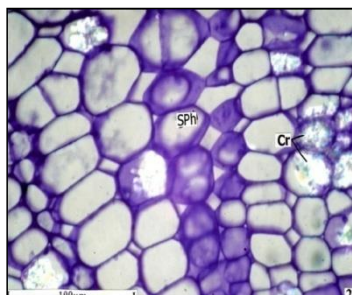


Figure 16

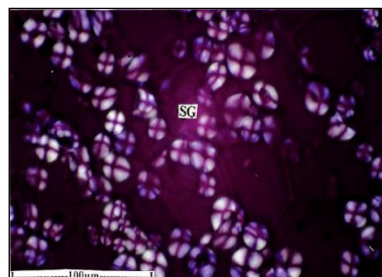


Figure 17

CO- Cortex; PE- Periderm; SPH- Secondary Phloem; SX- Secondary Xylem; CR- Crystals; SG- Starch Grains; XR- xylem Ray

Figure 14: T.S of root ground plan; Figure 15: T.S of root showing starch grains in the xylem ray; Figure 16: Crystals in the secondary phloem; Figure 17: Starch grains enlarged.

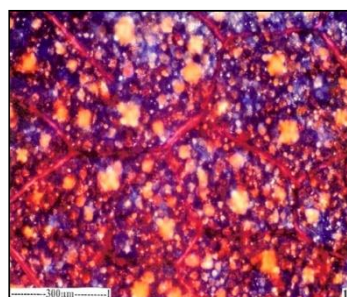


Figure 18

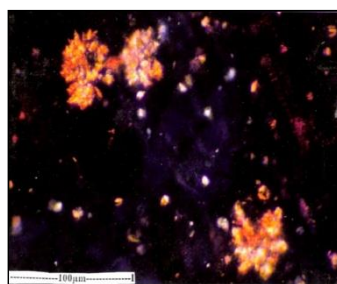


Figure 19

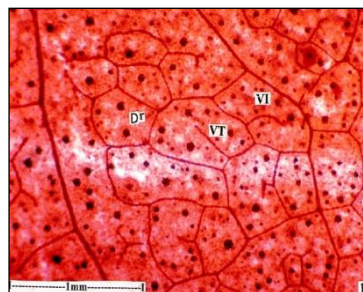


Figure 20

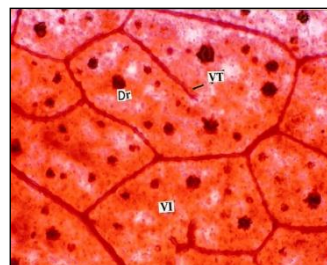


Figure 21

DR- Druses; VI- Vein-Islets; VT- Vein-Termination

Figure 18: Cleared leaf showing druses in the mesophyll tissue; Figure 19: Druses enlarged; Figure 20: Cleared leaf showing vein-islets and vein termination; Figure 21: Enlarged vein termination.

The xylem cylinder was cleaved radially into 8-10 radial segments with dilated xylem rays in between the segments. Periderm comprises of 4 to 6 layers of thin walled, tubular, suberised cells and 100 µm in thickness. Cortex was 300 µm wide, large, tangentially elongated, subdivided secondary phloem occurs in thick blocks on the outer ends of the xylem segments. They had small diffusely distributed

sieve elements and wider parenchyma cells. Secondary xylem consists of wide, angular, fairly thick walled solitary vessels embedded in narrow, thin walled xylem parenchyma cells. Xylem rays were wide and extend into the phloem, further wide in tangential plane. The ray cells were polyhedral parenchyma cells with dense accumulation of starch grains. The center of the root was densely crowded, with

narrow xylem elements and thick walled xylem fibers. The central core of the xylem had a tetrarch primary xylem elements (Figure-14).

Cell inclusions: Starch grains and calcium oxalate crystals were the dominant cell inclusions. In the root, crystals were located in the cortical cells and phloem parenchyma (Figure-15 & 16). Crystal druses were large, solitary and occupy entire lumen of parenchyma cells (Figure-16). The crystal ball was 25 µm wide. Starch grains of 15-20 µm wide were more abundant in the xylem rays (Figure-14). The crystals were simple, circular and concentric. They exhibit thick “+” mark when they were viewed under the polarized light microscope (Figure-17)

Powder microscopy:The powder of the leaf and root shows some diagnostic features. Calcium oxalate druses were abundant in the leaf powder. They occur in singles in a mesophyll cell. The druses were associated with the minor veins of the lamina (Figure-19). The druses were scattered freely in the powder as dense masses (Figure- 18 & 19). Small fragments of lamina were also seen in the powder. They show distinct vein islets and vein terminations (Figure- 20 & 21). The vein terminations were long and slender, either straight and unbranched or curved and forked

CONCLUSION

Pharmacognostic studies of *Talinum cuneifolium* enabled the developing of histological standards of the botanicals as a raw materials and its characterization. This stands a substantive basis for differentiation. It is further a fundamental avenue to ascertain the genuine and authentic samples from the adulterated samples. The generated data ultimately provides a basis for application in developing herbal monograph and for standardizing the raw materials used in formulation of therapeutics. The microscopy of leaf of *Talinum cuneifolium* has prominently projecting midrib. Midrib is narrow, deep and has adaxial groove. Mucilage filled idioblasts are frequently found in the midrib ground tissue and tanniferous cells are also occasionally seen in cells. It contains xylem and phloem. The lamina is amphistomatic (stomata on both upper and lower sides). The epidermal cell walls are fairly thick and

smooth. No cuticular striations are evident. The stomata are predominantly paracytic type on both sides of the lamina. There is a pair of subsidiary cells, one on either side of the stoma and the subsidiary cells are unequal size. They are oblong and lie parallel to the guard cells. The guard cells are 15x20 µm in size. The young stem has an intact epidermis, narrow cortex, central pith and a circle of about 14 discrete vascular bundles. The root consists of a narrow superficial periderm, wide cortex and a circular solid cylinder of vascular tissues. Starch grains and calcium oxalate crystals are the dominant cell inclusions. Starch grains are more abundant in the xylem rays and 15-20 µm wide. The crystals are simple and located in the cortical cells and phloem parenchyma. They are circular and concentric. The powder of the leaf and root shows some diagnostic features. Calcium oxalate druses are abundant in the leaf powder. They occur in singles in a mesophyll cell or quite uniquely, the druses are associated with the minor veins of the lamina. The druses are seen scattered freely in the powder as the dense masses. Small fragments of lamina are also seen in the powder. They show distinct vein islets and vein termination. The vein termination is long and slender, either straight and un-branched or curved, and forked.

Ethical Statement: This manuscript doesn't report any studies on animal experimentation.

Conflict of interest statement: Authors declare that there are no competing interests.

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