



## PHARMACOGNOSTICAL EVALUATION OF *TERMINALIA PALLIDA* BRANDIS

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### ABSTRACT

The present communication deal with the pharmacognostical studies of *Terminalia Pallida* Brandi's leaf. It is endemic plant of Tirumala hills and is commonly called as Tellakarka, Velamakaraka in Telugu. This plant has considerable economic importance and its fruits are recommended mainly for medicinal use. No systematic study reports are available on the pharmacognostical studies of the leaf. Hence, the present work was undertaken. The study revealed to study the various parameters which help in the identification of crude drug and adulteration of original drug with lower or inferior quality of the drug.

**Key words:** *Terminalia Pallida* Brandis, Medicinal plants, Pharmacognostical studies, Histochemical studies, Physical constants.

### INTRODUCTION

*Terminalia pallida* is endemic plant and belongs to family Combretaceae. Generally it is called as Tellakarka and Velamakaraka in Telugu, Vallaikkadukkay in Tamil and White gallnut tree in English. It is a taxonomically and phylogenetical complex group, consisting of 20 genera and 500 species of trees, shrubs and lianas distributed mainly in tropical and subtropical countries (Saik Abdul Latheef *et al.*, 2008). The Genus *Terminalia* Linn includes about 200 species of Trees and shrubs and distributed throughout the tropical and subtropical regions of the world. Besides yielding high value of timber, many *Terminalia* species are the source of various non-wood forest products (Yoganarasimham, 1950). 20 species have been found, distributed rarely in tropical and subtropical states of India i.e. Madhya Pradesh, Uttar Pradesh, Jammu, Karnataka, Kerala, Andhra Pradesh (Vedavathy, 1988). Its fruits are used in the treatment of diabetes (Anonymous, 1976).

As a powder applied externally on affected part and given orally with water to control diabetic and fruits are also consumed as dry pickles (Pallani *et al.*, 2009). As paste, mixed with turmeric and applied externally to the toes and feet to cure fissures and cracks in feet and in veterinary medicine. Fruits powder decoction is used to cure piles and diabetic (Savithramma, 2011). The leaves are used in indigenous drugs preparations, in many industries like pharmaceutical, animal husbandry, leather, dyeing, soap, chemical, resin, gum, paper, oil, cosmetics preparations etc and *Terminalia* species also utilized as source of raw materials (Johansen, 1940).

### MATERIALS AND METHODS

#### *Plant material*

The whole plant of *Terminalia pallida* Brandis leaves were collected freshly from Tirumala Hills near Talakona, Chittoor District, Andhra Pradesh, India and were authenticated by Dr.K.Madhava Shetty, Taxonomist, Sri Venkateswara University, Tirupati, Chittoor District, A.P, India. A voucher specimen was kept at S.V.University for further reference. The fresh leaves were used for present Pharmacognostical studies.

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**Pharmacognostical studies:** The pharmacognostical evaluation/studies like Macroscopy, Histology, physical constants, physicochemical constants, histochemistry, powder microscopy etc. were studied by using standard procedures laid down pharmacopoeias which help in the characterization and identification of crude drug.

**Histological studies:** Various histological characters have been studied by using standard procedure laid down in the standard books.

**Experimental Procedure:** The collected plant parts were fixed in FAA (Formalin 5 ml + Acetic acid 5 ml + 70% Alcohol 90 ml) in the field itself. Free-hand section, paraffin embedded sections and macerations were employed wherever necessary. Drawings were made with the use of prism type camera lucida and photomicrographs were taken using Nikon lab photo 2 microscopic unit. The plant materials were dehydrated in N-butyl alcohol (NBA) (Trease and Evans, 2006).

Then the cut sections and powdered drug were placed in dry clean slide and observed under Trinocular microscope (first 10X and then 40X) and microphotograph was taken by using Nikon Digital camera (labmod) (coolpix 4500 (4.0 mega fixed 4X Zoom) (Khandewal, 2008).

**Staining:** The staining is carried out by using toluidine blue 'O', Safranin 'O' and Fast green 'FCF'. Toluidine blue 'O' is a differential staining of sections was achieved without removing the paraffin by using 0.05% toluidine blue in water. After staining and washing in distilled water, the slides were dried, dewaxed in xylene and mounted in DPX. Stains are prepared by dissolving about 2.25 gm of safranin in 225 ml of 95% alcohol. 1 g of fast green was dissolved in a mixture of clove oil and absolute alcohol (in the ratio of 75:25). The dewaxed slides were stained in safranin for sufficient time and washed in alcohol until the excess stain was removed. Then the slides were stained in fast green and differentiated

**Vein-islets and vein terminations:** Collected fresh leaves are boiled in chloral hydrates solution in a test tube placed in boiling water. The soak in water, 10% Hydrochloric acid and chloralhydrate solution. Mount the preparation in glycerin-water. Put up the camera lucid and divide the paper in to square of 1 square millimeter by means of stage micrometer. Replace the stage micrometer by cleaned leaf preparation and trace the veins in four contiguous squares either in a square of 2x2mm or average of 1mmx4mm. Trace the vein islets and vein termination by looking through microscope when superimposed image of the leaf position and paper is seen at the same time. Count the number of vein islets and vein terminations present within the square or rectangle and also by taking into

consideration incomplete vein-islets on any two adjacent sides of the same square or rectangle (Khandewal, 2008).

**Histochemistry** (Khandewal, 2008): Microchemical tests and testing behavior of specific reagent towards plant drug tissue was carried out. The plant drug tissue was subjected to various reagents based up on the inference, the histochemical zones of the *Terminalia Pallida* leaves for the presence of various phytoconstituents i.e, tannins (Santha *et al.*, 2009), glycosides, saponins, alkaloids, anthraquinones, bitter principles were estimated. The reaction of powder with various reagents also studied (Dhiraj *et al.*, 2009; Kokate *et al.*, 1995)

**Physico-chemical constants:** The physicochemical constants like ash values, moisture constant, extractive values were determined by using standard procedure laid down in standard books (Anonymous, 1985, 1986).

**Ash values:** The leaf powder material used for the determination of total ash, acid insoluble ash, water soluble ash and sulphated ash of the *Terminalia Pallida* as per the procedure laid down in Indian pharmacopoeia.

**Total ash:** About 3gm of dried powdered material was accurately weighed and taken into previously ignited and tarred crucible. The powder was evenly spread as a fine layer and ignited gradually increasing the temperature to 450°C until it is devoid of carbon particles. The crucible was cooled in desiccator and weighed. The procedure was repeated to get a constant weight. The percentage of total ash was calculated with reference to air dried material (Patwardhan *et al.*, 2004).

**Acid insoluble ash:** The ash obtained as described in the above method was boiled gently with 25ml of 2N HCl for five minutes. The insoluble ash was collected on ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred into silica crucible and was ignited to a constant weight. The percentage of acid insoluble ash was calculated with respect to air dried drug.

**Water soluble ash:** To the crucible containing the total ash, 25 ml of distilled water was added and boiled for 5 min and filtered through ashless filter paper. No.41. The filter paper containing the insoluble matter was transferred into a silica crucible and ignited for 15 minutes at a temperature not exceeding 450°C and the procedure was repeated to get constant weight. Subtract the weight of the ash in milligrams from weight of the total ash to get water soluble ash. The percentage of water soluble ash was calculated with respect to air dried material.

**Sulphated ash:** Three grams of powdered drug was accurately weighed and taken in a silica crucible, ignited until the substance was thoroughly charred, cooled and moistened the residue with 1ml of sulphuric acid, heat gently until white fumes are no longer evolved and ignited at  $80 \pm 25^{\circ}\text{C}$  until all carbon particles have been disappeared. Cool the crucible and add few drops of sulphuric acid. Then ignited as before and further allowed to cool and weighed. The procedure was repeated until constant weight was obtained. The percentage of sulphated ash value was calculated with respect to air dried drug (Wermuth, 2004).

**Moisture content:** Weighed accurately 1.5gm of the powdered drug in to a pre- weighed flat and thin petridish and dried in an oven at  $105^{\circ}\text{C}$  for 45 minutes and cooled in desiccator and weighed. The procedure was repeated for constant weight and calculated the percentage of loss of moisture content of the crude drug.

## RESULTS

**Macroscopy:** Leaves (Fig.1) are ovate to broadly elliptic, coriaceous Emarginated oblong or roundish, glabrous, shortly petioled, leaf base rounded, variation Conduplicate. Leaf measures 2.5 – 5.0 cms x 3.08 – 1.3cms. Tastes slightly bitter and smell agreeable.

**Microscopy:** The leaf constants of *Terminalia pallida* like stomatal number, stomatal index, vein islet number, vein termination number and palisade ratio were determined by using standard procedures prescribed in pharmacopoeia. The results of these quantitative Microscopical studies were presented in Table.2.

**Physico- chemical** evaluation of *Terminalia pallida* leaves powder was studied like as total ash 18.60%, water soluble ash 7.26%, acid insoluble ash 8.48%, Sulphated ash 14.45% and loss on drying or moisture content is 4.20%. The extractive values of *Terminalia pallida* in petroleum ether is 3.60%, ethylacetate 8.66% and methanol is 14.30%. Values are mean of duplicate i.e.=2 results are expressed in percentage w/w.

**The Histochemical** tests of TP powder was determined by using different agents to identify the presence of various phytochemical constituents and observation and the results pertaining to the micro-chemical tests and behavior of specific reagents towards plant tissues are presented in the Table 3 and 4.

**Histological studies:** (Figure 2 to 11): T.S. of the petiole is circular in outline, shows epidermis ground tissue (cortex) and vascular bundle. Epidermis is single layered composed of thin walled Cubical Cells covered externally by thin cuticle and some of the epidermal cells elongate

from simple unicellular trichomes. Ground tissue is large consisting of 2 to 5 layers of Collenchyma and rest of the cells are thin walled to thick walled parenchymatous cells filled with abundant clustered calcium oxalate crystals. Some of the cells show simple to rounded starch grains. Vascular strand closed and dorsally flattened vascular bundle/strand are collateral conjoint and open encircled by well developed, ring of phloem fibers. Near the vascular strand, prominent abundant ring of clustered calcium oxalate crystals are present many layered, closely arranged thin walled rounded parenchymatous cells filled with clusters of calcium oxalate crystals are present in the central region of the petiole of vascular bundle.

**Midrib region** (Figure 12 to 22): Leaf presents a dorsiventral structure T.S through the mid rib region shows Plano convex structure, both upper and lower epidermis shows simple unicellular trichomes. Towards the adaxial side 2 to 4 layered collenchymas and 2 to 5 layered parenchyma cells contain clustered crystals of calcium oxalate. Towards the abaxial side 1 to 3 layered collenchyma and 1 to 4 layered rounded, thin to thick walled compactly arranged parenchymatous cells filled with clustered calcium oxalate crystals vascular bundle is closed and dorsally flattened with well-developed xylem, phloem and phloem fibers. Vascular bundles are enclosed by well-developed ring or circle of phloem fibers in 2 to 5 or more in groups and abundant ring of clustered calcium oxalate crystals. Vascular bundle is collateral, conjoint and open. Xylem is separated by uniseriate medullary rays and in the firm of continuous cylinder. Phloem cells are twin walled and many layered.

**Laminar region** (Figure 23 to 26): T.S. of the laminar region shows dorsiventral structure with well-developed upper and lower epidermis with two layered elongated palisade cells on both the side's i.e. upper & lower surface and loosely arranged spongy parenchyma. Cells of the palisade parenchyma and spongy parenchyma are brown in color cells of the Mesophyll tissue are filled with clustered calcium oxalate crystals and secretory cavities. The Vascular bundles of the veins are bicollateral and accompanied with sclerenchymatous. Leaf exhibits ranunculaceous type of stomata usually confined to the lower surface of the leaf.

### Diagnostic Characters

1. Presence of Dorsiventral leaf
2. Presence of Ranunculaceous type of stomata
3. Presence of stomata on the lower surface of the leaf only
4. Presence of abundant clustered calcium oxalate crystals in the ground tissue of the leaf petiole and mesophyll tissue.

5. Presence of prominent ring of clustered calcium oxalate crystals near the vascular bundle of petiole and mid rib region.
6. Presence of ring of prominent phloem fibers in groups 2 to 5 or more near the vascular bundle of petiole and midrib region
7. Presence of secretory cells / resin cells in the laminar region
8. Presence of simple starch grains in the ground tissue of petiole
9. Presence of unicellular simple trichomes
10. Presence of ramifying sclerenchymatous fibers of vascular bundles of the veins
11. Presence of 2 layers of palisade tissue on both the sides of leaf that is both on upper and lower surface.

**Powder Microscopy:** The powder microscopy was carried out by sieving the drug with 60 mesh and then powder placed on the slide and treated with chloral hydrate solution and water. The different fragments /tissues were observed under the microscope (Patwardhan *et al.*, 2004).

**Microscopy of TP leaf powder** (Fig 27 to 35): Powder is green in color smell agreeable taste - slightly sweetish with mucilaginous feeling. When treated with chloralhydrate and water observed under the microscope different fragments of tissues were seen.

**Table 1. NButyl alcohol dehydration series**

Sl. No	Ethyl alcohol 95% (ml)	NBA (ml)	Distilled water (ml)	Time in hours
1	20	10	70	1
2	25	15	60	1
3	30	25	45	1
4	30	40	30	1
5	25	55	20	2
6	20	70	10	2
7	15	85	-	2
8	-	100	-	2

**Table 2. Leaf constants of Terminalia Pallida leaf**

Name of the plant	Leaf constant	Values
<i>Terminalia Pallida</i> leaf	S.N. lower epidermis	5-6-7
	S.N. upper epidermis	6-7-9
	S.I. lower epidermis	12.31 – 14.21 – 16.35
	S.I. upper epidermis	6.25 – 7.35 – 8.35
	Vein islet Number	6-7-8
	Vein termination Number	14 – 20 – 24
	Palisade ratio	15 – 20 – 25

**Table 3. Histochemical tests of Terminalia Pallida leaf**

S.No.	Reagent	Test for	Color	Result
1	Iodine solution	Starch	Blue	++
2	10%FeCl <sub>3</sub> solution+ alcoholic)	Tannin	Black	++
3	Sudan III Solution	Oil globules	No Change	--
4	Chloral hydrate	Crystals	Clear	++
5	Phloroglucinol HCl + alcohol	Lignin	Magenta	++

++ → Positive / Present, -- → Negative / Absent

**Table 4. Reaction of T.P leaf powder with different reagents.**

S.No.	Test	Colour
1	Powder + Water	light Green
2	Powder + Sudan III Solution	No Change
3	Powder + Iodine Solution	blue Color
4	Powder + 10% FeCl <sub>3</sub> Solution	black Color
5	Powder + 10 % NaOH Solution	No change
6	Powder + Con. H <sub>2</sub> SO <sub>4</sub> Solution	black Color

Figure 1. Terminalia Pallida leaf twig with flower buds and leaf.



Figure 2. Macroscopy of leaves



Figure 3. T.S of the petiole



Figure 4 & 5. Petiole portion enlarged

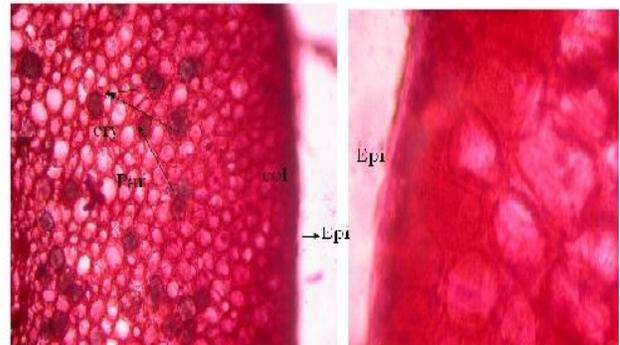


Figure 6. Parenchyma & Figure 7. Crystals & collenchymas spongy tissue

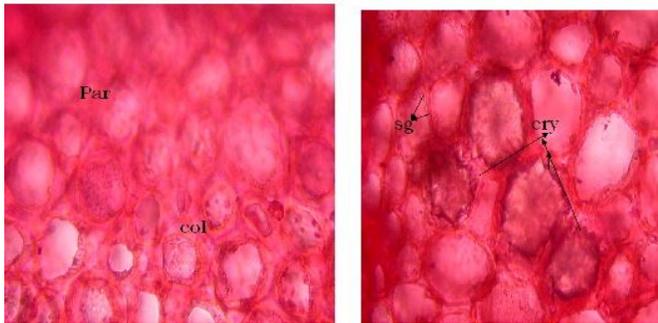


Figure 8. Cortex showing crystal & Figure 9. Vascular bundles

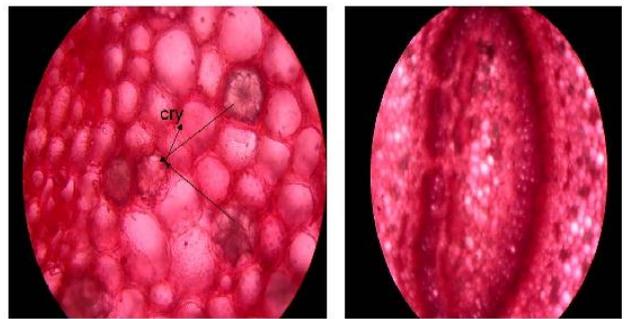


Figure 10. Vascular bundles enlarged & Figure 11. Calcium oxalate crystals

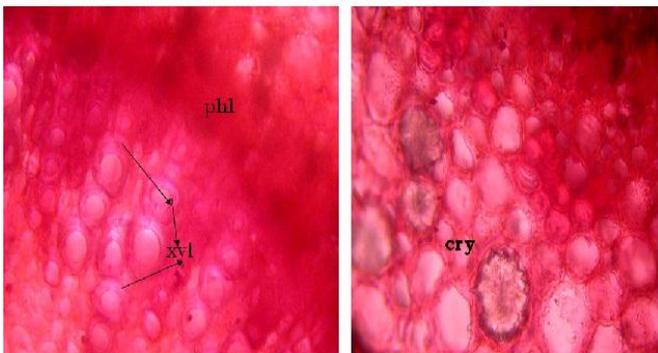
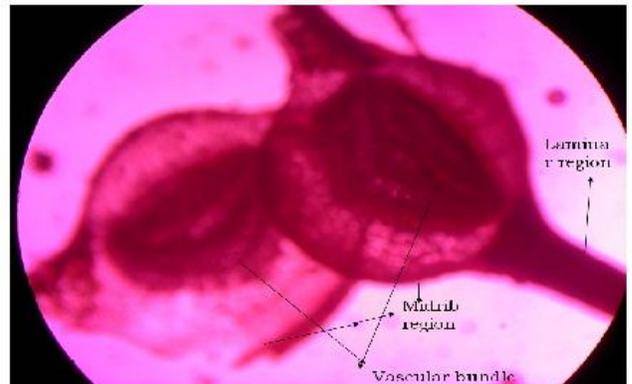
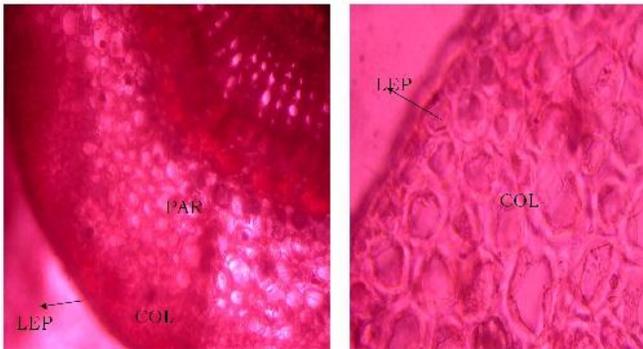


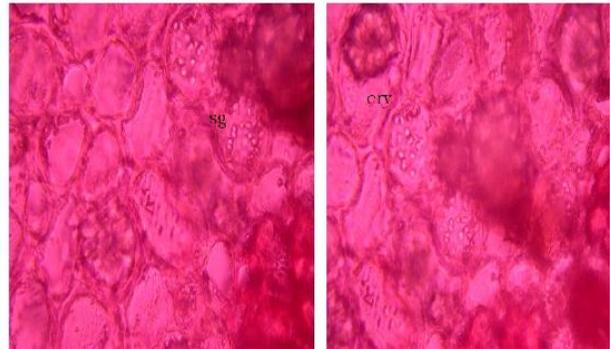
Figure 12. T.S of the leaf



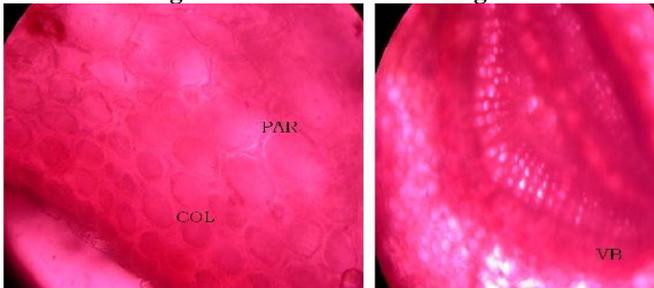
**Figure 13. Portion enlarged & Figure 14. Ground tissue**



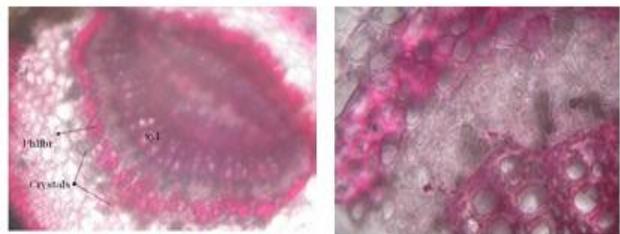
**Figure 15. Starch grains & Figure 16. Crystals**



**Figure 17 & 18 Portion enlarged**



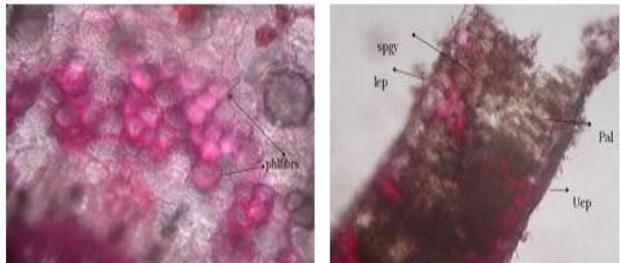
**Figure 19. Vascular bundle & Figure 20. Vascular bundle enlarged**



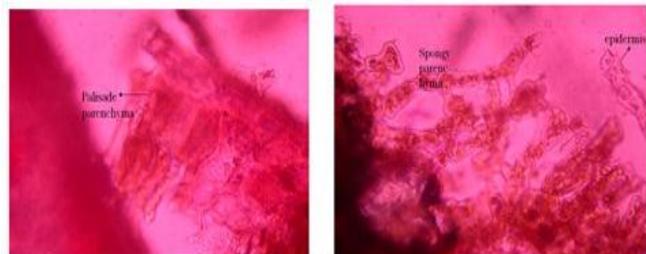
**Figure 21. Enlarged portion showing & Figure 22. Xylem enlarged xylem, phloem and phloem fibers**



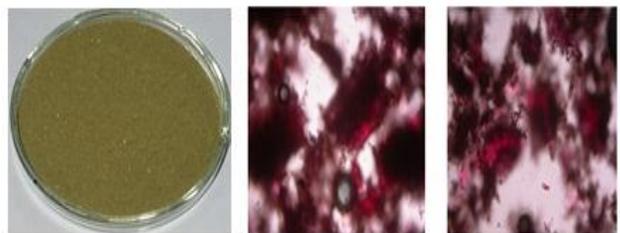
**Figure 23. Phloem fibers enlarged & Figure 24. Laminar region enlarged**



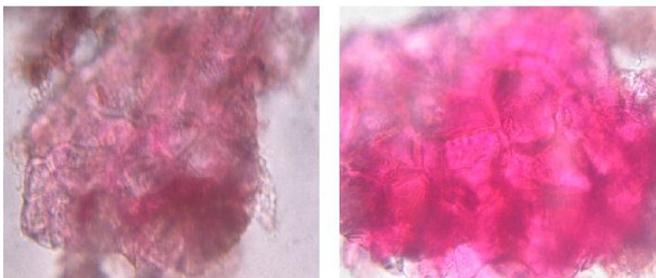
**Figure 25. Laminar portion enlarged & Figure 26. Mesophyll enlarged**



**Figure 27&28. Powder macroscopy and fragments of tissues of TP**

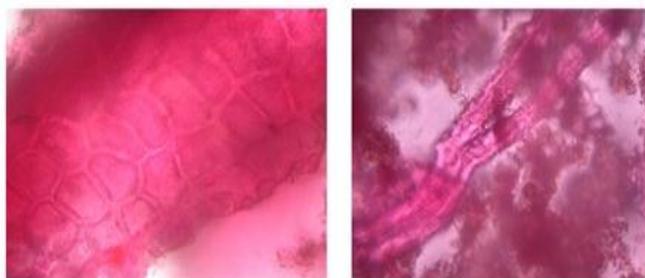
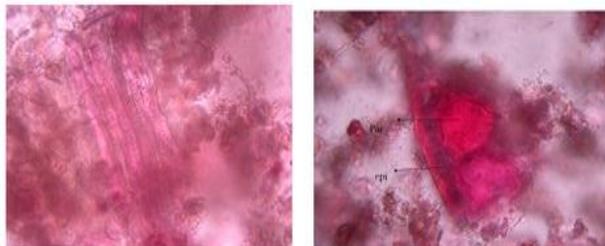


**Figure 29. Fragments of parenchyma cells with tannin**



**Figure 30. Crystal & Figure 31. Trichome**



**Figure 32. Epidermal cells & Figure 33. Fibers****Figure 34. Fragments of xylem & Figure 35. Fragments of epidermal and helical vessels and parenchyma cells**

## DISCUSSIONS

Ethno-pharmacology and drug discovery using natural products remain important issues in the current target-rich and lead-poor scenario (Wermuth, 2004). Many modern drugs have their origin in ethno-pharmacology. Globally, there is a positive trend in favor of traditional and integrative health sciences in both research and practice. There are common approaches to drug discovery including the use of chemical biology, serendipity, chemical synthesis, combinatorial chemistry and genomics. However, the innovative approaches involve ethno-pharmacology, reverse pharmacology, holistic, system biology and personalized medicine. There are clear trends to show that the mainstream in pharmaceutical research is moving away from single molecule or single target approach to combinations and multiple target approaches (Raghunath, 2005). The ethno-pharmacology knowledge and experimental base allows drug research from clinics to laboratories - a true reverse pharmacology approach. In this process, 'safety' remains the most important starting point and the efficacy becomes a matter of validation. A golden triangle consisting of traditional knowledge, modern medicine and modern science with systems orientation will converge to form an innovative discovery engine for newer, safer, affordable and effective therapies (Dve, 1997).

An analysis of the origin of the drugs developed between 1981 and 2002 showed products of natural product-derived drugs comprised 28% of all new chemical entities launched into market (Newmann et al., 2000). In addition, 24% of these new chemical entities were synthetic or natural mimic compounds, based on the study of pharmacophores related to natural products. This combined percentage (52% of all new chemical entities) suggests that natural products are important sources for new drugs and are also good lead compounds suitable for further modification during drug development.

Pharmacognostical studies are useful in developing histological standards of raw materials and its characteristics, by which means, we can differentiate, the genuine and authentic samples from the adulterated samples (Dhiraj, 2009). This would help in developing

herbal monograph and standardize the raw materials used in the formulation.

**Histology:** (Figure 1 to 11) Histological characters of *Terminalia pallida* shows the presence of dorsiventral leaf, ranunculaceous type of stomata, stomata present only on the lower surface of the leaf, abundant clustered calcium oxalate crystals in the ground tissue of the leaf petiole and mesophyll tissue, presence of prominent ring of clustered calcium oxalate crystals near the vascular bundle of petiole and midrib region, ring of prominent phloem fibers in groups 2 to 5 or more near the vascular bundle of petiole (Fig 1 to 11) and midrib region, secretory cells / resin cells in the laminar region, simple starch grains in the ground tissue of petiole, of unicellular simple trichomes, ramifying sclerenchymatous fibers of vascular bundles of the veins and presence of 2 layers of palisade tissue on both the sides of leaf (Fig.12 to 26) i.e both on upper and lower surface helps for the identification of the plant from the other plants.

**Physical constants:** Quantitative physical Constants of *Terminalia Pallida* like stomatal number, stomatal index, vein-islet number, vein-termination number and palisade ratio were determined by using standard procedures prescribed in pharmacopoeia. These leaf constants are the diagnostic characteristics of crude drug (Sing and Gvil, 2002) and are constant to the particular medicinal plant helps in identification of the crude drugs. These results are presented in Table. 2. Micro chemical tests of TP also indicate the presence of starch, tannins, crystals, steroids, triterpenoids, glycosides and proteins. These Histochemical tests/reactions (3 and 4) will help to identify the various chemical constituents present in a particular medicinal plant (Santha et al., 2009).

*Terminalia pallida* contains physico-chemical constants like ash content (Table No5.7) it is due to the presence of high inorganic content. However the ash content is may be due to the presence of sodium and calcium salts which are not harmful. It is also constant to the medicinal/ herbal drugs and is useful to identify the quality of the herbal drugs. The ash value in the crude

drug is more than the extracts. The values of the plants were within the limits. Ash value is the measure of the quality and purity of crude drug. The moisture content helps in determining the storage conditions of crude drugs which may spoil either due to chemical change or microbial contamination.

The extractive values give an idea of the amount of phytoconstituents eluted in the respective solvent and helps to estimate the percentage of yield (Harborne and Mabry, 1982). The extractive values are constant to the particular medicinal plants.

**Powder study** (Figure 27 to 35): Macroscopically leaf powder is dark green in colour, small agreeable, tastes slightly bitter, when powder is treated with chloral hydrate solution and water, observed under the microscope, The following fragments of tissues were observed. Macroscopy of TP leaf powder drug, different fragments of tissues, fragments of parenchyma cells with tannin, calcium oxalate crystals, trichomes, epidermal cells, fibers, fragments of xylem, fragments of epidermal and helical vessels, fragments of epidermal and parenchyma cells

## SUMMARY AND CONCLUSION

The Indian systems of medicines like Ayurveda, Siddha derive more than 85% of the drugs from plant source. Drugs came in to existence from very early times to alleviate pain and cure the diseases. Their utility as substances to overcome body disorders gave them the little drugs. Drugs play an important role in Ayurveda, Siddha and other traditional systems of medicine. The identification of drug hence is of paramount importance for the survival of these systems of medicines. Standardization of drug in Ayurveda play a major role in the identification based on the scientific parameters like taxonomical, pharmacognostical and phytochemical characters,. The several endomorphic characters found in the plants help in the pharmacognostical identification, while phytochemical studies provides parameters for identification of the drugs in powder form.

The *Terminalia Pallida* has different Macroscopical characters like ovate leaves, glabrous, ovate rounded leaves, Microscopical characters like

secretary cells, in the laminar region simple starch grains in the ground tissue unicellular simple trichomes, ramifying sclerenchymatous fibers of vascular bundles in veins, 2 layers of palisade tissue on both upper and lower side of the leaf ring of prominent phloem fibers in groups of 2 to 5 or more near the vascular bundle of petiole and midrib region permanent ring of clustered calcium oxalate crystals near the vascular bundle of the petiole and mid rib region. All the diagnostic characters will help in the identification of the drug *Terminalia pallida* from other species of *Terminalia*. Even in powder form also all the microscopic characters that are found in fragmented form, will help in the identification of the drug in powder form from different species.

Thus Pharmacognostical studies of *Terminalia Pallida* revealed that both macro and Microscopical studies plays a major role in the identification of different species and it is of paramount importance in the present study in the helps for the students research scholars in identification of the different species an also in revalidation of the plant species. Likewise powder study with different reagents also plays a key role in the identification of drug in powdered form. The different color that is observed by treating the powder with different reagents is also one of the important characters which will indicate in identification of the drug in powdered form.

The different quantitative studies that in carried out is also one of the important key factor in the identification of the drug .The stomatal number various considerably with the age of the leaf and due to changes in environmental conditions, whereas stomatal index is relatively constant and therefore of diagnostic significance for a given species. Hence the stomatal index is employed for the differentiation of allied or closely related species of the same generous in air dried as well as, fresh conditions. Hence a quantitative study also plays a role in the identification of drug. In over all the studies carried out *Terminalia pallida* are of much use in the identification of drugs and also traditionally the plant has been attributed to have some medicinal values in treating different diseases and also the drug claimed to have folklore uses.

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