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Pharmacognostic standardization of *Pimpinella tirupatiensis* bal. & Subr. an endemic to eastern ghats, Tirumala hills, Andhra Pradesh

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Abstract
To evaluate the pharmacognostic characters of *Pimpinella tirupatiensis Bal. & Subr.* leaf, stem, and root tuber. Pharmacognostic parameters like macroscopic, microscopic and powder study of this species were undertaken. Preliminary phytochemical, physiochemical analysis and fluorescent behaviour were done by using reported methods. The macroscopic study showed that the leaf was simple, deeply cordate, veins prominent. The microscopic study of leaf revealed dorsiventral type of cellular arrangement, multicellular trichomes, anisocytic stomata. The microscopic study of stem shown conjoint, collateral, open and endarch vascular bundles. Root tuber revealed diarch type of xylem. Physiochemical analysis of leaf showed total ash, water soluble ash and acid insoluble ash. In all parts, maximum extractive value was high in water than alcohol soluble. Various pharmacognostical characters would help in identification, standardization, quality control and formulation development of *P. tirupatiensis.*

Keywords: Fluorescence analysis, pharmacognosy, preliminary phytochemical screening, powder behaviour, *Pimpinella tirupatiensis*

Introduction
Plants have been used as treatment for thousands of years, based on experience and folk remedies and continue to draw wide attention for their role in the treatment of mild and chronic diseases. In recent times, focus on plant research has increased all over the world and a large body of evidence has been accumulated to highlight the immense potential of medicinal plants used in various traditional systems of medicine. *Pimpinella tirupatiensis* Bal. & Subr. (Family Apiaceae; local name, kondakothimera) is a rare and endemic medicinal plant and restricted to the Seshachalam hills of the Eastern Ghats, India (Balakrishnan & Subramanyam 1960) [2]. It is an erect herb with perennial tuberous root stock, Stem is simple, branched, branches alternate, bifurcate, veins prominent, reddish brown on lower surface, margins cartilaginously crenate - serrate. Leaves are simple, ovate, obtuse or acute, deeply cordate, petiolate. Flowers are white, compound umbles, Fruits ovoid, papillose-scrabrous. The tuber consists of a tap root arising from a stout root stock which persists throughout the life of the plant. The boiled tubers of *P. tirupatiensis* used as food. This plant is prescribed for venereal diseases and peptic ulcers (Nagaraju and Rao, 1989) [12]. The local Adivasi tribal (Erukalas, Nakkalas, Sugali, Yanadis) community uses the tuberous roots of *P. tirupatiensis* to cure severe ulcers of stomach, throat and genital organs and also as aphrodisiac and abortifacient agents. Fruits are used to cure asthma and are considered as an effective remedy for 'flatulent colic' (Thammanna and Narayana Rao, 1990). Dried roots of *P. tirupatiensis* are administered along with few other ingredients to cure colic and rheumatic ailments in cattle (Sudarsanam *et al*., 1995) [18]. This plant root extract is also used to treat skin diseases and is used as an antimicrobial agent (Jeevan Ram *et al.*, 2004) [8]. The whole plant of *P. tirupatiensisis* used to treat cough, stomach, liver problems, asthma, ulcer and toothache (Madhava Chetty *et al*., 2008) [8]. The objective of the present study is to evaluate various pharmacognostical parameters such as macroscopic, microscopic, physicochemical, fluorescence and phytochemical studies of the plant.

Materials and methods
Collection and Authentication:
The plant material *Pimpinella tirupatiensis* was collected from Japaliteertham, Tirumala hills, Chittoor district, Andhra Pradesh. The taxonomic identity of the plant is confirmed by Dr. K. Madhava chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati and voucher specimen was (Pt 2207) preserved in the herbarium, Plant taxonomy lab, Division of Botany, Department of Sericulture,

Sri Padmavathi Mahila Visvavidyalayam (Women's University), Tirupati, Chittoor District for future reference. Plant parts like tubers root, stem, and leaf were taken for the anatomical study and the plant parts are preserved in 70% alcohol; Some plant parts were washed thoroughly with tap water, shade dried, homogenized to fine powder and stored in air tight bottles for further use.

Pharmacognostic study

Macroscopic study

Fresh leaf, stem, and root tuber was collected from Japaliteertham, Tirumala hills, Chittoor district, Andhra Pradesh, India. The macro morphological feature of the leaf, stem and root tuber was observed under magnifying lens (Tyler *et.al.*, 1977) [19].

Microscopic characteristics

The microscopic evaluation was done by taking free hand thin sections which were prepared for each part taken and stained as per standard procedure by using safranin to confirm lignification and fast green and I+KI (for starch). Various identifying characters such as trichomes and cell composition were recorded and then pictomicrography was done. Powder microscopy of dried leaf, stem, and root tuber powder was studied under microscope. The anatomical structures and cell components were observed and their photographs were taken by using phase contrast microscope.

Qualitative phytochemical analysis

The crude powder of leaf, stem and root tuber was subjected to qualitative phytochemical analysis (Kokate, 1986 and Harborne, 1973) [10, 7]. The phytochemicals analysed were alkaloids, flavonoids, tannins, steroids, saponinis, phenolic compounds and anthocyanin dins.

Physicochemical analysis

The physicochemical analysis of the crude powder *P. tirupatiensis* leaf, stem and root tuber was carried out as per WHO guidelines. The parameters analysed were Loss on drying, Total Ash, Water soluble ash, Acid insoluble ash, Alcohol soluble extractive, Water soluble extractive values.

Fluorescence analysis

Fluorescence study of leaf, stem, and root tuber powder was performed as per reported standard procedures (Kokoski, *et.al.*, 1958) [9]. A small quantity of the leaf/stem/root tuber powder was placed on porcelain spot plate grease and 2-3 drops of freshly prepared reagent solution was added, mixed by gentle tilting of the slide and waited for few minutes. Then the slide was placed inside the UV chamber and observed in visible light, short (254 nm) and long (365 nm) ultra violet radiations. The colour observed by application of different reagents in different radiations was recorded.

Statistical analysis

All experiments were repeated at least three times. Results are reported as Mean ± S.E.M. (Standard Error of Mean).

Results

Organoleptic and macroscopic characteristics

The organoleptic features of *P. tirupatiensis* leaf, stem and root tuber.

It is an erect herb with perennial tuberous root stock, 70-85 cm tall. Stem is simple, branched, branches alternate, bifurcate, branch lets glabrous, veins prominent, reddish brown on lower surface, margins cartilaginously. Leaves are simple, ovate, obtuse or acute, deeply cordate, leaf margins contain anthocyanin pigments, measures 3-4cm long and 4-5cm diameter, petiole 2.4 – 5.5 cm long, cauline leaves palmately 3-partite. Flowers are white, 5-12, in compound umbles bracteoles 1-2, very small, linear. Petals long, glabrous, styles small, slender, 1mm long, dithecous anthers, 1mm long, stylopodium conical, 1mm long, yellowish brown, persistent. Fruits 1.5 mm long, ovoid. The tuber consists of a tap root arising from a stout root stock which persists throughout the life of the plant. It is a thick and fleshy. It becomes tapering and grows inside the soil up to about 50 cm. The tubers occur in carrot like pieces of varying length, measures 19-25 cm long and 5-7 cm diameter. (Fig: 1)

Fig 1: (a) *Pimpinella tirupatiensis* Habitat (b) *P.tirupatiensis* tubers (c) *P. tirupatiensis* whole plant (d) *P.tirupatiensis* leaf margins containing anthocyanin pigments (e) & (f) *P.tirupatiensis* anther two views (g) stylopodium (h) bilocular ovary (i) *P. tirupatiensis* seeds.

Microscopic characteristics of leaf of *P. tirupatiensis* Leaf

A transverse section of leaf *P. tirupatiensis* through the midrib region shows three main parts called epidermis, mesophyll and vascular bundles. The epidermis on both sides of the leaf is made up of a single row of barrel shaped cells that are arranged closely and compactly without intercellular spaces. Both sides of epidermis are covered by a thick cuticle. The epidermis also shows multicellular hairs which are uniseriate. The number of stomata is more in the lower epidermis than the upper epidermis. Mesophyll was differentiated into upper palisade and lower spongy parenchymatous cells. Palisade tissue is composed of one layer of closely arranged elongated cells. The palisade tissue in the mid-rib region is replaced by collenchymatous tissue. It is present above the vascular bundle and gives the mechanical strength. The lower half of the mesophyll tissue occupied spongy parenchyma cells. The cells are irregular, thin walled having intercellular spaces. Vascular bundles are conjoint, collateral and closed. Xylem is present towards the upper epidermis and phloem is towards the lower side. Tannin and oil cells are seen in both upper and lower epidermis and anisocytic stomata were also present (Fig: 2). the stomatal index of upper surface and lower surface is 21.0 ± 2.23; 23.9 ±1.39. The vein islet number is 22.3±1.15 and veinlet termination is 12.6±1.52. The palisade ratio is 13.6 ± 0.57. Fig: 2(a).

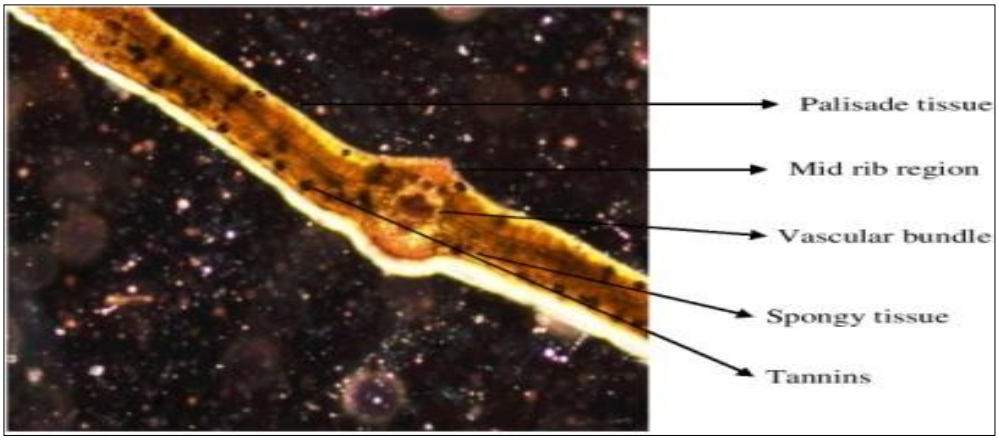
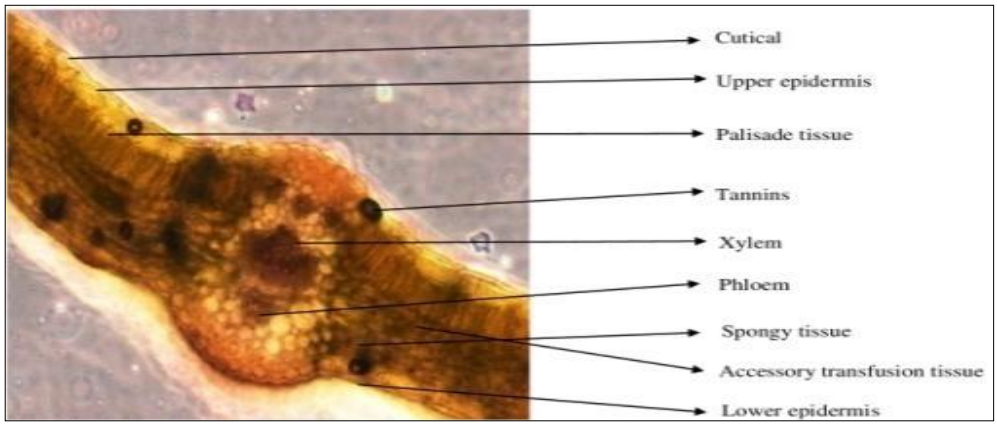


Fig 2(a)



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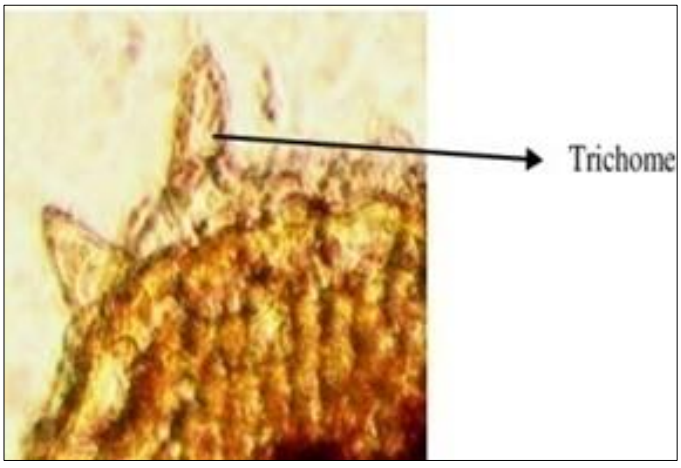


Fig 2(c)

Fig 2(a): Transverse section of leaf *P. tirupatiensis*
Fig 2(b): Transverse section of leaf *P. tirupatiensis*

Powder study of leaf of *P. tirupatiensis*

The crude powder of leaf was dark green in colour with characteristic odour and aromatic taste. The powdered leaf of *P. tirupatiensis* under microscopic investigation showed epidermal cells, uniseriate, multicellular trichomes, anisocytic stomata, spiral xylem vessels slightly lignified, lignified fiber, simple starch grains, tannin and oil cells. Fig: 3.

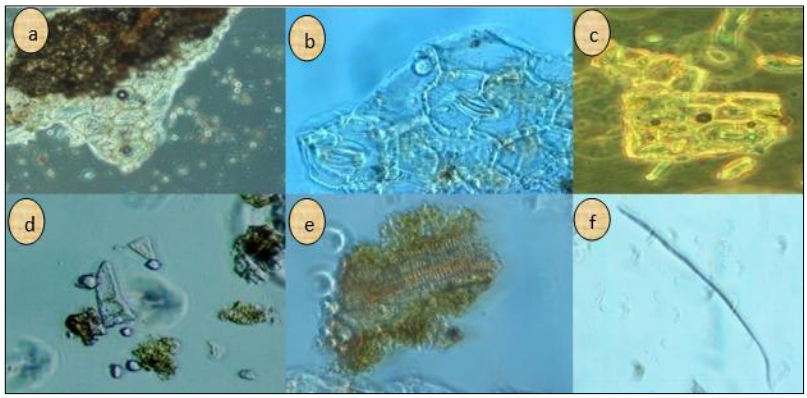
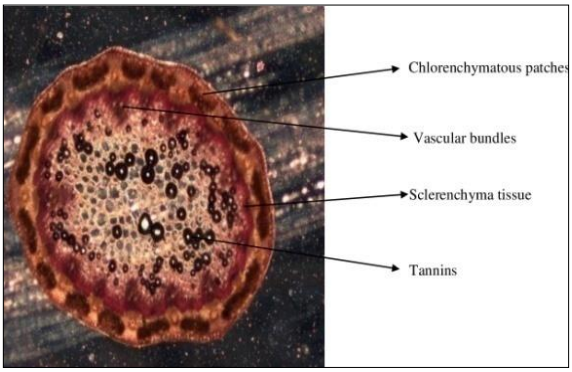


Fig 3(a): Epidermal cells with tannin and oil cells (b) Anisocytic stomata (c)Parenchyma cells with stomata and tannin cells (d) Uniseriate, Multicellular trichomes with simple starch grains (e) Spiral xylem vessels slightly lignified (f) Lignified fibers.

Microscopic studies of stem of *P. tirupatiensis* Stem

A cross section of young stem shows slightly ridges and furrows. The epidermis consists of a single layer. It is covered by a thick cuticle on the outer side. Collenchyma is prominent below the ridges. The cortex is made up of parenchyma, chlorenchymatous patches. Resin ducts are prominently seen in cortex region. Endodermis and pericycle are not very distinct and is made up of thin walled cells. Vascular bundles are conjoint, collateral, open and endarch. Sclerenchyma tissue cells are 4–5–seriate between two peripheral vascular bundles. Medulla contains more tannins and parenchymatous cells (Figure: 4)



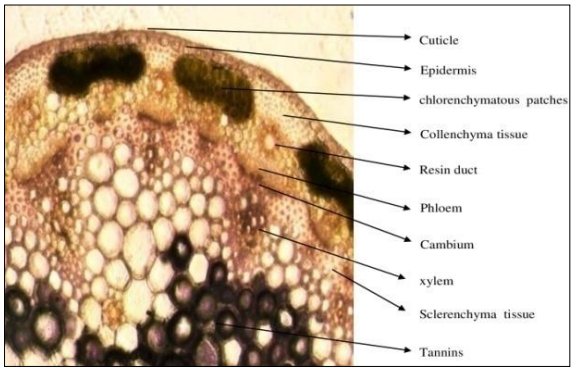


Fig 4(a) Fig: 4(b)

Fig 4(a): Transverse section of stem *P. tirupatiensis*
Fig 4(b): Transverse section of stem *P. tirupatiensis* (A Sector enlarged)

Powder study of stem of *P. tirupatiensis*

The crude powder of stem was light green in colour. The stem powder of *P. tirupatiensis* under microscopic investigation showed parenchymatous cells, resin duct, various shaped calcium oxalate crystals, xylem elements, lignified fibers, scalariform vessels, simple starch grains, tannin and oil cells (Fig: 5).

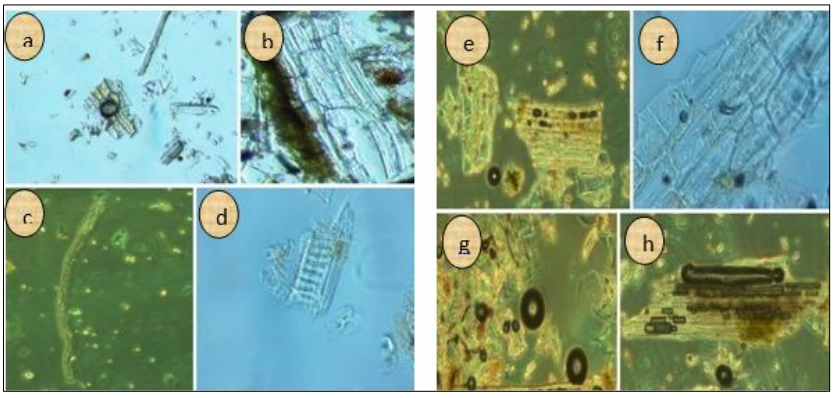


Fig 5(a): Resin duct with various shaped calcium oxalate crystals(b) Xylem elements (c) Lignified fiber (d) Scalariform vessels (e) Parenchyma cells with bunches of tannin and oil cells (f) Parenchyma cells with tannins (g) Bunches of calcium oxalate, oil& tannin cells (h) Xylem fibers.

Microscopic studies of root tuber of *P. tirupatiensis* Root tuber

A transverse section of the root tubers shows the following regions. The outer most region is the epidermis. Next to the epidermis are 8-10 layers of tangentially elongated cells which constitute the cork. Next to the cork is a very large cortex made up of many layers of parenchymatous cells. Some cells towards the upper region contain oil. Some cells of cortex contain sparsely distributed starch grains, prism and rod shaped calcium oxalate crystals. Next to the cortex is stelar region consisting of diarch type of xylem. In between the xylem, medullary rays are found interspersed which travels a major portion of the cortex and gets diffused. In between the medullary rays, phloem is found (Fig:6). The endodermis and pericyle are indistinct. The salient and diagnostic features of the *P. tirupatiensis* tubers are presence of oil cells towards the upper region of the cortex, rod shaped calcium oxalate crystals in the cortex region, sparsely distributed simple starch grains, stellar region with diarch xylem.

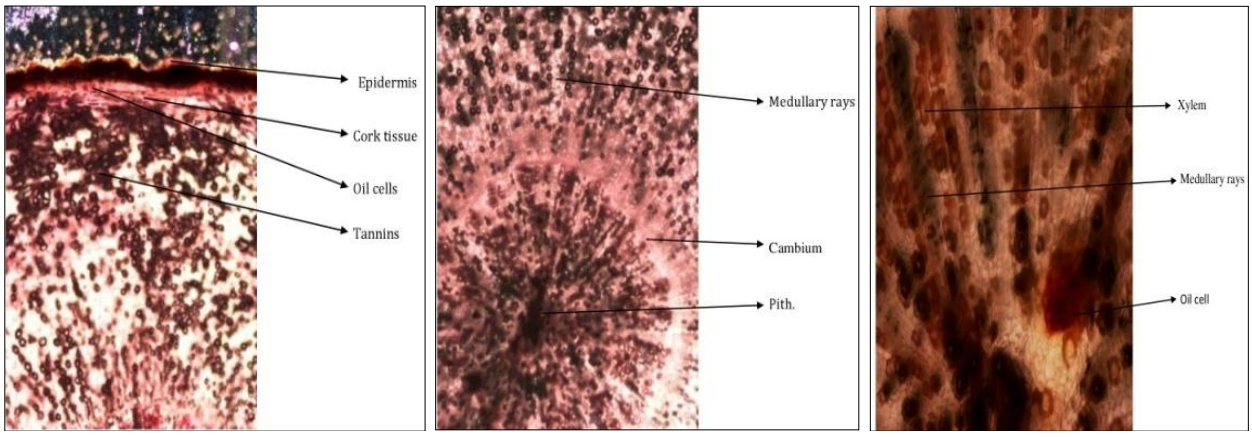


Fig 6(a) **Fig 6(b)** **Fig 6(c)**

Fig 6 (a), (b), (c): Transverse section of root tuber *P. tirupatiensis*

Powder study of root tuber of *P. tirupatiensis*

The crude powder of root tuber was light brown in colour. The root tuber powder of *P. tirupatiensis* under microscopic investigation showed cork cells, simple starch grains, various xylem vessels, prism, rod shaped calcium oxylate crystals, medullary rays, xylem fiber, tannins and oil cells (Fig: 7).

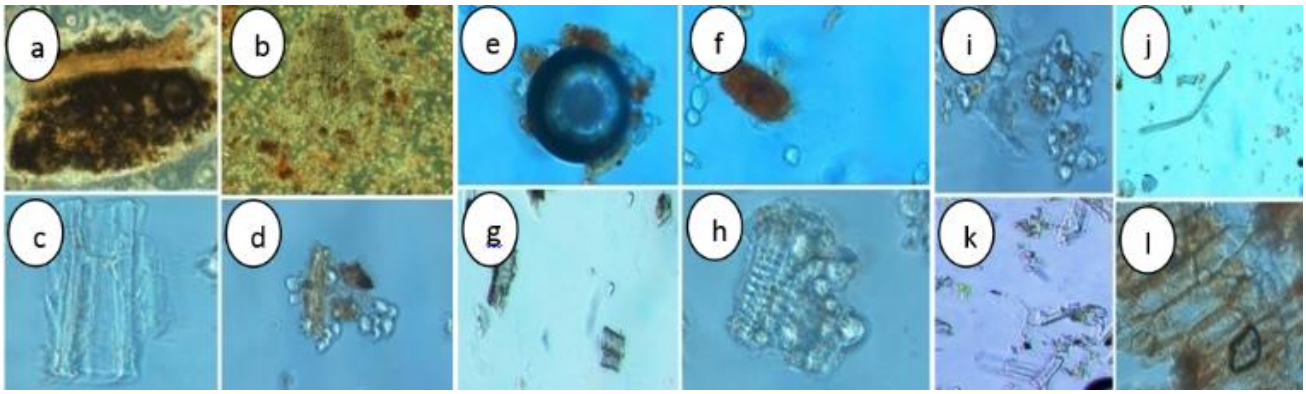


Fig 7: (a) cork cells with oil& tannin cells (b) Simple starch grains with tannin & oil cells(c) Xylem vessels (d) Parenchymatous cells with simple starch grains (e) Tannin cell (f) Oil cell (g) scalar form vessels (h) Spiral xylem vessels with simple starch grains (i) Simple starch grains (j) Xylem fiber showing lumen & various rod, prisum shaped crystals (k) various types of Calcium oxylate crystals (l) Medullary rays.

Phytochemical analysis

The results of the qualitative phytochemical analysis of the crude powder of *P. tirupatiensis* leaf, stem and root tuber are shown in Table: 1. The leaf has shown the maximum amount of tannins followed by alkaloids, flavonoids and anthocyanin dines, while stem has shown the maximum amount of tannins followed by flavonoids, and alkaloids. The root tuber has shown the maximum amount of alkaloids followed by tannins. The other phytoconstituents like flavonoids, steroids, phenolic compounds and saponins were present.

Preliminary phytochemical screening of *P. tirupatiensis* Leaf, Stem, and Root tuber.

Table 1: Phytochemicals present in less amount (+), moderate (++) high amount (+++) and absent (-)

S. No	Name of Phytochemical Test	Leaf	Stem	Root tuber
1	Alkaloids	+++	+++	+++
2	Flavonoids	++	+++	+++
3	Tannins	+++	++	++
4	Steroids	-	+	++
5	Phenolic compounds	+	++	-
6	Saponins	++	++	++
7	Anthocyanin dins	++	+	+

Physicochemical Analysis

The physiochemical characterization of *P. tirupatiensis* leaf, stem and root tuber are shown in Table 2. The moisture content of leaf, stem, and root tuber observed was 8.5, 7.8 and 8.8 % respectively. The ash value was determined by three different forms viz., total ash, water soluble ash and acid insoluble ash. The total ash in leaf was 10%, while water soluble ash and acid insoluble ash was 1.9 and 3.0 % respectively. The total ash in stem was 9.6% while both water soluble ash and acid insoluble ash was 1.7% and 2.63% respectively. The total ash in root tuber was 9.5%, while water soluble ash and acid insoluble ash was 1.9 and 2.7 % respectively.

Table 2: Physicochemical parameters of *P. tirupatiensis* Leaf, Stem and Root tuber.

S. No	Parameters	% Value (w/w*) Leaf	% Value (w/w*) Stem	% Value (w/w*) Root tuber
1	Loss on drying	8.5 ± 1	7.8 ± 0.57	8.8 ± 0.28
2	Total ash	10 ± 0.5	9.6 ± 0.28	9.5 ± 0.5
3	Water soluble ash	1.9 ± 0.057	1.7 ± 0.1	1.9 ± 0.057
4	Acid insoluble ash	3.0 ± 0.1	2.63 ± 0.30	2.7 ± 0.2
5	Aqueous soluble extractive value	12.4 ± 0.115	12.1 ± 0.230	12.5 ± 0.503
6	Alcohol soluble extractive value	1.17 ± 0.0115	1.12 ± 0.011	1.09 ± 0.023

Fluorescence analysis

The fluorescence characteristics of leaf, stem, and root tuber of *P. tirupatiensis* were analyzed. The fine powder was added with different acids, bases, and was observed under visible, short UV wavelength (254nm), and long UV wave length (365nm), it showed different colour variation (Fig:8), presented in Table 3. Fluorescence study helps in the qualitative evaluation which can be used as a reference data for the identification of adulterations.

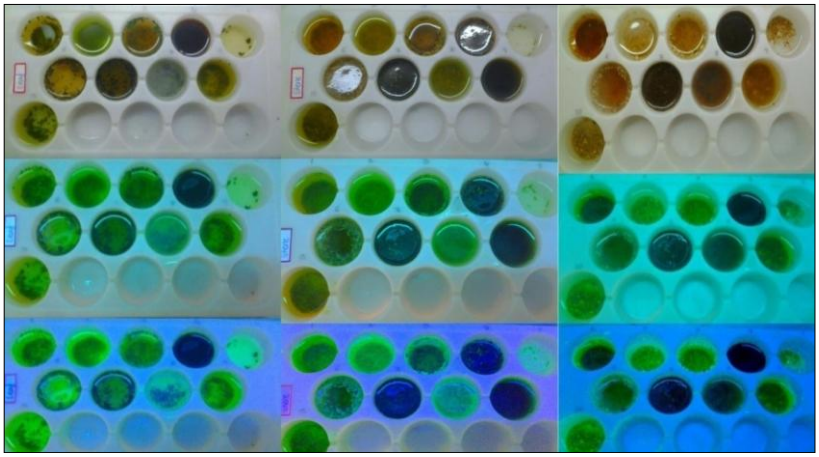


Fig 8: Fluorescence analysis of powder of Leaf, Stem, Root tuber of *P. tirupatiensis* by using various solvents.

Table 3: Fluorescence analysis of powder of Leaf, Stem, Root tuber of *P. tirupatiensis*

Treatment	Plant Part	Observations		
		Visible light	Short UV Wavelength(254nm)	Long UV Wavelength(365nm)
Powder + 1 N NaOH (aq)	Leaf	Light Brown Colour	Light Green Colour	Green Colour
	Stem	Light Brown Colour	Light Yellow Green Colour	Fluorescence Light Green Colour
	Root tuber	Brown Colour	Light Green Brown Colour	Fluorescence Green Black Colour
Powder + 1 N NaOH (alc)	Leaf	Green Colour	Green Colour	Green Colour
	Stem	Light Green Colour	Light Yellow Green Colour	Green Colour
	Root tuber	Brown Colour	Green Colour	Green Colour
Powder + Ammonia	Leaf	Light Brown Colour	Dark Green Colour	Green Colour
	Stem	Light Brown Colour	Green Colour	Light Green Colour
	Root tuber	Brown Colour	Green Colour	Green Colour
Powder +Picric acid	Leaf	Black Colour	Black Colour	Black Colour
	Stem	Balck Colour	Black Colour	Black Colour
	Root tuber	Black Colour	Black Colour	Dark Black Colour
Powder +Petroleum ether	Leaf	Light Green Colour	Light Green Colour	Fluorescence Light Green Colour
	Stem	-----	Light Green Colour	Light Green Colour
	Root tuber	Black Colour	Green Colour	Green Colour
Powder + 50% HCl	Leaf	Light Brown Colour	Light Green Colour	Green Colour
	Stem	Brown Colour	Green Colour	Green Colour
	Root tuber	Light Brown Colour	Dark Green Colour	Green Colour
Powder + 50% H2SO4	Leaf	Brown Colour	Green Colour	Green Colour
	Stem	Black Colour	Black Colour	Black Colour
	Root tuber	Black Colour	Black Colour	Black Colour
Powder + Ethyl acetate	Leaf	----	Green Colour	Light Green Colour
	Stem	Black Colour	Black Colour	Dark Black Colour

	Root tuber	Brown Colour	Black Colour	Light Black Colour
Powder + Ethyl alcohol	Leaf	Light Brown Colour	Green Colour	Green Colour
	Stem	Dark Green Colour	Green Colour	Light Green Colour
	Root tuber	Light Brown Colour	Light Green Colour	Green Colour
Powder + Methanol	Leaf	Light Green Colour	Light Yellowish Green Colour	Green Colour
	Stem	Light Green Colour	Light Yellow Green Colour	Green Colour
	Root tuber	Light Brown Colour	Green Colour	Green Colour

Discussion

Standardization is very important and essential to maintain the identity, quality, purity and safety of crude drugs especially in the powder form. Pharmacognostic and physicochemical parameters must be determined. The morphological characters can serve as diagnostic characters of a particular plant species and will be very useful in identifying the plant at species level and prevent adulteration.

In this regard, the microscopic and macroscopic features of leaf, stem and root tuber have been studied. Studies revealed the presence of anisocytic type of stomata, trichomes, xylem vessels, tannins, oil cells etc. In stem vascular bundles are conjoint, collateral, open and endarch. In root tuber simple starch grains, rod shaped calcium oxalate crystals, oil cells, tannin cells, diarch type of xylem are observed which are the characteristic features of *P. tirupatiensis Bal.& Sub*. (Apiaceae family). Studies of physicochemical constants can serve as a valuable source of information and also useful in judging the purity and quality of the drug. The extractive values give an idea about the chemical constitution of the drug and from the study, it is observed that the extractive values of leaf, stem and root tuber are higher in water content compared to alcohol. The ash value determines the earthy matter or inorganic composition and other impurities present along with the drug. Total ash value is higher in leaf followed by stem, and root tuber. Water soluble ash and Acid insoluble ash are higher in leaf followed by root tuber and stem. Similar results are observed by Sudhakar *et al.*, (2011) [17] who have carried out macro and microscopic features as well as Preliminary phytochemical test, Physico-chemical and fluorescence analysis of root tuber of *P. tirupatiensis.*

Conclusion

The Pharmacognostical study which includes macroscopy, microscopy, powder behaviour, fluorescence studies and phytochemical analysis gives valuable information. This is essential for correct identification and diagnostic tool for the standardization of this medicinal plant which would be helpful in the characterization of the crude drug.

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