

## Research Article

# Pharmacognostical studies on the root tuber of *Pimpinella tirupatiensis* Bal. & Subr. - An endemic to Tirumala Hills of Eastern Ghats, India

A. Sudhakar\* C. Ramesh, N. Nagaraju and K. Sri Rama Murthy<sup>1</sup>

*Herbal Folklore Research Centre, Department of Botany,  
Sri Venkateswara Arts College, Tirupati - 517 502, Andhra Pradesh, India*

<sup>1</sup>*School of Conservation Biology and Plant Biotechnology,  
Department of Biotechnology, Montessori Mahila Kalasala,  
Vijayawada 520 010, Andhra Pradesh, India*

### ABSTRACT

*Pimpinella tirupatiensis* Bal. & Subr. (Apiaceae) has potential medicinal value in traditional medicine for various use like aphrodisiac, stomachache, sexual debility, ulcers in the stomach, mouth, throat etc., The present study deals with the Pharmacognostical examinations like morphological and histological characters of root tubers of *Pimpinella tirupatiensis*. besides physiochemical, fluorescence and phytochemical analysis. These observations will enable to standardize the botanical identity of the drug in its crude form. Data evolved in this exploration could be used in laying down pharmacopoeial principles for the drug studied, as standardization of herbal medicines is completely essential and is need of the hour.

Keywords: Pharmacognosy, *Pimpinella tirupatiensis*, root tuber.

### INTRODUCTION

India is an emporium of medicinal and aromatic plants. It has been estimated that out of 15,000 higher plants occurring in India, 9000 are commonly used, of which 7500 are used in various systems of medicine<sup>1</sup>. *Pimpinella tirupatiensis* Bal.& Subr. (Apiaceae) is an erect herb with perennial tuberous root. It is a seasonally occurring and narrowly distributed endemic taxon in Tirumala hills of Seshachalam ranges in Southern Eastern Ghats<sup>2</sup>. The tuberous root is used as an aphrodisiac and also to allay stomachache<sup>3</sup>. Infusion of tuber is pounded with coconut, sugar, cashew nut and boiled in cow's milk, is taken orally as halva (sweet product) twice a day to cure sexual debility. Tuber powder is also mixed with honey and taken orally to cure ulcers in the stomach, mouth, throat and genital system. Raw tuber is given to terminate 2 to 3 month pregnancy<sup>4,5,6,7</sup>. Hence the present study was under taken to develop Pharmacognostical parameters such as morphology, histology, ash values, extractive values, fluorescence analysis and preliminary phytochemical studies for establishing the Pharmacopoeial standards and botanical identity of *Pimpinella tirupatiensis*.

### MATERIALS AND METHODS

#### Collection and Authentication

Fresh tuberous of the plant *Pimpinella tirupatiensis* was collected from wild source in the Tirumala hills. The plant was identified and authenticated with the help of local floras. Final identification was confirmed with reference to authentic specimen available in Madras Herbarium, Coimbatore (MH). A voucher specimen was

deposited (AS 121) in the Herbal Folklore Research Centre (HFRC), Department of Botany, S.V. Arts college, Tirupati, Chittoor district for future reference. The fresh tubers were made into free hand and microtome sections about 10-12  $\mu$ m. The shade dried tubers were powdered for phytochemical physio-chemical and fluorescence studies.

Table 1. Measurements of different cells and tissues of *Pimpinella tirupatiensis*

Tissue	Measurement ( $\mu$ )
Tuber (T.S.)	
Cork	12-20-32 x 25-40-48
Parenchyma (cortex)	20-24-80x 16-32-40
Xylem	40-56-71x 20-28-36
Phloem	20-28-36x8-16-20
Starch grains	8-16-24 (diameter)
Macerate	
Parenchyma	12-24-60x 8-12-16
Vessel	100-120-200 x 20-32-48
Starch grains	12-20-32 (diameter)

#### Pharmacognostical Studies

The organoleptic characters such as color, odour, taste, size, shape and surface of the tubers of *Pimpinella tirupatiensis* were studied according to the standard procedure<sup>8,9</sup>. A paraffin embeded tubers were sectioned with the help of rotatory microtome at the three different levels. The thickness of the section was 10- 12 $\mu$ m. De-waxing of the section was done by customary procedure<sup>10</sup>. The sections were stained with toluidine blue as per the method published by (O'Brien et al)<sup>11</sup>. Wherever necessary sections were also stain with safranin

\*Author for Correspondence

Email: [sudhakarnaidubotany@gmail.com](mailto:sudhakarnaidubotany@gmail.com)

Table 2. Histochemical tests for tuber of *Pimpinella tirupatiensis*

Drug	Reagents	Test for	Reaction	Results
Section	Iodine solution	Starch	Blue colour	++
Section	Ferric chloride solution	Tannin	Light black colour	+
Section	Sudan III solution	Oil globules	Pink colour	+
Section	Con. HCl	Crystals	Effervescence (rod shaped crystal)	+
Section	Pinch of phloroglucinol + dil. HCl + alcohol	Lignin	Magenta colour (xylem region)	+

and fast green and I+KI (for starch). Microscopic descriptions of the tissue are supplemented with photomicrograph wherever necessary. For normal observation bright field was used and for the study of crystal, starch grains and lignified cell, polarized light was employed<sup>12</sup>.

#### Phytochemical Analysis

The tubers were collected, washed with water, dried under shade and powdered by using wearing blender and passed through sieve no 60. Coarse powder was used for various phytochemical investigations. Ash value of the powdered drug was determined by using standard procedure in a silica crucible<sup>13</sup>. The dried powder drug was extracted with various solvents in the order of increasing polarity. The different extracts were filtered and concentrated under vacuum. The extractive

values were also calculated by standard procedure<sup>14</sup>. The powdered drug as well as various extracts was analyzed for fluorescence studies under visible light and UV 254nm. The extract of the tubers were subjected to qualitative chemical tests for the identification of various active constituents<sup>14-18</sup>.

#### RESULTS AND DISCUSSION

##### Morphology

Erect herb with perennial tuberous root stock, 30-100 cm tall. Stem simple below, branched above, terete, striate; branches alternate, bifurcate; branchlets glabrous, veins prominent, reddish brown on lower surface, margins cartilaginously crenate - serrate. Basal leaves simple, ovate – obtuse or acute, deeply cordate 1.7 - 3.8 x 1.3 – 3.8 cm, petiole 2.5 – 7.8 cm long, sheathing

Table 3: Phytochemical screening of extracts of *Pimpinella tirupatiensis*

Test	Benzene	Ethanol	Hexane	Chloroform
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Indoles	-	-	-	-
Leucoanthocyanins	-	-	-	-
Steroids	+	+	-	-
Carbohydrates	+	+	+	+
Phenols	+	+	+	+
Steroid nucleus	-	-	-	-
Proteins	-	+	+	
Lignins tannins		+		
Saponins	+	+	+	+
Terpenoids	+	+		+
Methylenedioxy functional compounds	+	+	+	+
Coumarins	+	+	+	-

at base, cauline leaves palmately 3-partite. Flowers white, 5-16, in compound umbles; bracteoles 1-2, very small, linear. Petals 1 mm long, glabrous, obovate, sub-orbicular, apex inflexed; styles small, slender, 1mm long; stylopod conical, yellowish brown, persistent. Fruit 1.5 mm long, ovoid, papillose - scabrous.

Table 4: Physico-chemical constant of *Pimpinella tirupatiensis*

Parameter	Values (% W/W)
Ash Values	
Total ash	9.50
Water soluble ash(%w/w)	1.75
Acid insoluble ash(%w/w)	3.00
Extractive values	
Water	12.0
Ethanol	0.9
Methanol	17.92
Ethyl acetate	12.10
Chloroform	2.4
Benzene	12.2

#### Organoleptic Characters

The tuber consists of a tap root arising from a

Table- 5: Fluorescence analysis of *Pimpinella tirupatiensis*

S.No	Particulars	Visible light	UV light 254nm
1	Alcoholic extract	Pale yellow	Fluorescence pale green
2	2 ml Alcoholic extract + Aqueous Ag NO <sub>3</sub> (1 drop)	--	Fluorescence green
3	2 ml Alcoholic extract + 0.1 N aqueous NaOH (3 drops)	--	Dark green
4	2 ml Alcoholic extract + 0.1N aqueous NaOH (2 drops)	--	Fluorescence green
5	Powder as such	Pale yellow	Fluorescence yellow
6	Powder + 1 N NaOH in methanol	--	High yellow fluorescence
7	Powder + 1 N NaOH in H <sub>2</sub> O	--	Green
8	Powder + 1 N H Cl	--	Fluorescence blue
9	Powder + 50 % HNO <sub>3</sub>	--	Purplish green
10	Powder + 50 % H <sub>2</sub> SO <sub>4</sub>	--	Light green

stout root stock which persists throughout the life of the plant. It is thick, fleshy, fusiform, tapering and grows inside the soil up to about 50 cm. the tubers occur in

carrot like pieces of varying length, measured to 30 cm long and 8 cm diameter, grows either straight or at time curved. It is brownish in colour and the surface in the upper portion exhibits ring like formation. The upper two-third portion is rough while lower one-third is smooth to touch. The root bark is very thin and peels off easily. A transverse section of a root shows a brownish stelar region surrounded by a large white parenchymatous cortex. It breaks off easily and had a characteristically pungent smell when cut open. Taste is slightly bitter and mucilaginous. Cut surface of dried root is light cream in colour. Dried tubers shrink and break off with granular fracture (Plate -1)

#### Microscopical Characters

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 paranchymatous cells. Some cells towards the upper region contain oil. Some cells of cortex contain sparsely distributed starch grains 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. The endodermis and pericycle 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, stelar region with diarch xylem (Plate - 2).

#### Powder Microscopy

The powder microscopy of the tubers shows the presence of thin walled parenchymatous cells which contain simple starch grains and found in groups; vessels are of various size and shape-drum shaped, barrel shaped with some drawn out ends. The measurements of various cell and tissues are provided respectively (Table - 1)

#### Histochemical Tests

The sections of the materials were treated with different reagents for the presence of starch, tannin, oil globules, crystals and lignin (Table -2).

#### Preliminary phytochemical test

Preliminary phytochemical test for hexane, benzene, chloroform and alcohol extract of the drug were carried out<sup>15</sup>. It shows the presence of alkaloids, flavonoids, indoles, leucoanthocyanins, steroids, carbohydrates, phenols, steroidal nucleus, proteins, lignins tannins, saponins, terpinoids, methylenedioxy functional compounds and coumarins (Table -3).

#### Physico-chemical Constants

The tuber powder was studied for their physico-chemical constants which include ash values, successive extractive values and solubility. It shows higher ethanol and chloroform extractive values 23.04%w/w and 23.34% w/w respectively (Table -4).

Fluorescence analysis of extract and drug powder

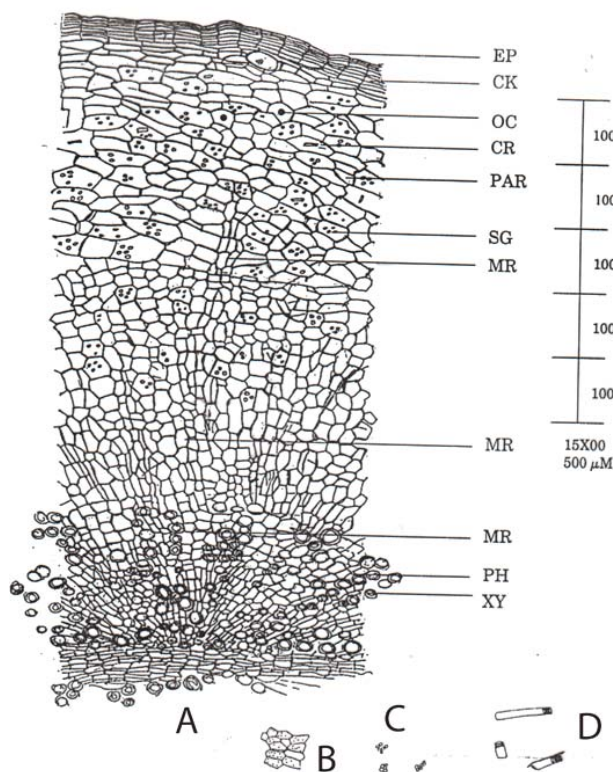


Figure 1. Microscopic characters of the root tuber of *Pimpinella tirupatiensis*

- A. T.S. showing the details of cell structures
- B. Macerate – Parenchyma with starch grains
- C. Macerate – Simple starch grains
- D. Vessles

Abbreviations : EP-epidermis; CK- cork; OC- oil cell; CR- crystal; PAR- parenchyma; SG- starch grain; MR- medullary ray; PH- phloem; XY- xylem

Fluorescence analysis of drug powder and its various extract treated with acid, alkali was studied and tabulated<sup>19</sup>. It shows yellowish for the powder drug and yellowish green fluorescence for ethyl acetate extract under white and visible light (Table-5).

The pharmacognostic study on the tubers of *Pimpinella tirupatiensis* has brought to the light of certain microscopic feature as well as preliminary phytochemical data of diagnostic values. Physico-chemical constants such as solubility, ash values, successive extractive values and other parameter such as fluorescence analysis and preliminary phyto-chemical studies are corroborate evidence in drug standardizations.

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