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# PHARMACOGNOSTICAL AND PHYTO-CHEMICAL EVALUATION OF PUNARNAVADI MANDURA: AN EFFECTIVE FORMULATION FOR IRON DEFICIENCY ANEMIA

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**ABSTRACT**
Iron deficiency anemia affects millions of people worldwide. Children and women of reproductive age are at increased risk. Iron deficiency is harmful at all ages. In young children it impairs physical growth, cognitive development, immunity and at school age it affects school performance. At adulthood it causes fatigue, reduced work capacity and in pregnant women, anemia leads to fetal growth retardation, low birth weight and maternal death. Punarnavadi Mandura Vati is a herbo-miniral formulation indicated for the management of Pandu (Anaemia). Pharmacognostical evaluation of Punarnavadi Mandura Vati exposed acicular crystals, sclerides, beaker shape Stone cell, pitted vessels, starch with parenchymal cells, resin contents etc. which are the characteristics of the drug. Organo-leptic features of coarse powder were within the standard range. High- Performance Thin Layer Chromatography Study showed 5 spots corresponding to $hR_f$ values 0.30, 0.66, 0.70, 0.76, 0.85 in short wave UV 254 nm, and 2 spots corresponding to $hR_f$ values 0.31, 0.69 obtained in long wave UV 366 nm.
**Keywords:** Pandu, Anaemia, Punarnavadi Mandura Vati, Pharmacognosy, Physio-chemical Analysis, High Performance Thin Layer Chromatography (HPTLC)

## INTRODUCTION

Iron deficiency anemia (IDA) is a global public health problem and harmful as the epidemics of infectious diseases. With a global population of 6,700 million, at least 3,600 million people have iron deficiency and 2000 million out of these suffer from IDA. Children and women in reproductive age group are more vulnerable. India continues to be one of the countries with the highest prevalence of anemia. National Family Health Survey (NFHS 3) estimates revealed the prevalence of anemia to be 70-80% in children and 70% in pregnant women. Indian Government started a National Anemia Prophylaxis Programme in 1970. Subsequently the programme was modified and renamed the National Anemia Control Program (NACP) in 1991 for Control and Prevention of anemia in women of reproductive age and pre-school children. These programmes have been operational for over 40 years, but have made a little dent on the overall prevalence of anemia. Recently, Government of India in collaboration with WHO, UNICEF and FOGSI launched the 12 by 12 initiative, on 23 April 2007[1]. Several other programmes focusing on issue of anemia includes: ICDS, Mid-day meal programme, Kishori Swasthya Yojna, Matri Suraksha Abhiyan, IMA Anemia free India, as a Public Private Partnership and Anemia Chale Jao etc. However, most of these programmes have not anticipated success and anemia prevalence goes on increasing.

Nutritional deficiency is considered to be the main etiological factor in IDA though the other factors like malabsorption, worm infestations, chronic diseases and hemorrhage are also mentioned. Pallor is the major symptom described in IDA. According to the Ayurvedic classics, sign and symptom of *Pandu Roga* are very much similar to iron deficiency anemia. Clinical features of Pandu develop from the depletion of Rasa Dhatu which in turn becomes ineffective in the production of Rakta Dhatu. The decreased level of circulating Rasa and Rakta Dhatu, which have the prime functions of nourishment and providing support to the vital functions, gives rise to the symptoms like depletion of blood and flesh, fatigue, body ache, palpitation, periorbital edema, anorexia, dyspepsia, fever, dyspnoea and fainting[2]. There are various herbal and herbo-mineral formulations mentioned in Ayurveda classics for the management of Pandu. Punarnavadi Mandura Vati contains Deepana, Pachana, Raktavardhaka and Ushana Virya Drugs. All are easily available. Most of them are Kaphavatashamaka. It contains Triphala which is a well known digestive rejuvenator, Trikatu which corrects the metabolism and Haridra scrapes out the unnecessary fat and other toxins out of the body. The identification and authenticity of ingredients through pharmacognosy and pharmaceutical chemistry measures is inescapable ladder needed for the quality assurance and standardization of any of the herbal medicine whether it is single drug or formulation. Punarnavadi Mandura Vati is the most potential Herbo-miniral preparation which is claimed by various researches to be extremely successful in the management of Pandu. It is an attempt to evaluate pharmacognostical and physico-chemical analysis of Punarnavadi Mandura Vati to prove its purity and authenticity.

### Aims and objectives

Pharmacognostical study and Phyto-chemical analysis of Punarnavadi Mandura Vati

## MATERIALS AND METHODS

### Procurement of raw material

All herbal parts of formulation were procured from the pharmacy, Gujarat Ayurved University, Jamnagar. Gomutra was collected from local areas of Jamnagar, Gujarat. The ingredients and the parts used are mentioned in table 1.

### Pharmacognostical Evaluation

Raw drugs were identified and authenticated by the Pharmacognosy Laboratory, I.P.G.T& R.A., Gujarat Ayurved University, Jamnagar. The identification was carried out based on the morphological, organolaptic and powder microscopy of the individual drug. Pharmacognostical evaluation of the tablet was also carried out. Tablet was dissolved in small quantity of distil water and filtered through filter paper. The filtrate was studied under the Corl zeiss microscope attached with camera, with stain and without stain. The microphotographs were taken under the microscope.

### Method of Preparation of the Punarnavadi Mandura Vati

The ingredients 1 to 20 (table 1) were cleaned, dried, powdered and pass through sieve number 85. The powder and Mandura bhasma were mixed with cow urine and heated. 5 % gum acacia was mixed as a binding agent. The mixture was converted in to granules with the help of granular machine and finally punched as tablets (500mg) by tablet making machine.

### Pharmaceutical Evaluation

### Physicochemical parameters

Punarnavadi Mandura Vati was analyzed by using standard qualitative and quantitative parameters at the pharmaceutical chemistry Laboratory, I.P.G.T. & R.A., Gujarat Ayurved University, Jamnagar. Parameters were selected on the basis of common parameters mentioned for compressed tablets in Ayurvedic pharmacopoeia of India[3] and CCRAS guidelines[4]. The formulation was assessed for total ash value, pH, water & alcohol soluble extracts and Iron Content. Presence of more moisture content may create preservation problem. Hence loss on drying was also selected as one of the parameter.

### High-Performance Thin Layer Chromatography study

Methanol extract of *Punarnavadi Mandura Vati* was spotted on pre coated silica gel GF 60 aluminium plate as 5 mm bands, 5 mm apart and 1 cm from the edge of the plates, by means of a Camang Linomate V sample applicator fitted with a 100 µL Hamilton syringe. Toluene (5ml), Ethyl acetate (4.5ml), Acetic acid (0.5ml) was used as the mobile phase. After development, Densitometric scanning was performed with a Camag TLC scanner III in reflectance absorbance mode at 254 nm and 266 nm under control of win CATS software (v1.2.1 camag). The slit dimentions were 6 mm x 0.45 mm and the scanning speed was 20mm per second. All HPTLC plates were scanned with filter faction Savitsky-goloy 7, minimum spot-5, minimum height 10AU, minimum area 50AU, maximum height 990 AU with absorption unit.

**Table 1: Ingredients of Punarnavadi Mandura**

| S.N. | Ingredients | Botanical name | Part used | Ratio |
|---|---|---|---|---|
| 1. | Punarnava | *Boerhaavia diffusa* Linn. | Root | 1part |
| 2. | Sunthi | *Zingiber officinalae* Roxb. | Rhizome | 1part |
| 3. | Trivrita | *Operculina turpethum* Linn. | Root | 1part |
| 4. | Maricha | *Piper nigrum* Linn. | Fruit | 1part |
| 5. | Pippali | *Piper longum* Linn. | Fruit | 1part |
| 6. | Vidanga | *Embelia ribes* Burm | Fruit | 1part |
| 7. | Devdaru | *Cedrus deodara* Roxb. | Kastha | 1part |
| 8. | Chitraka | *Plumbago zeylenica* Linn. | Root | 1part |
| 9. | Kushtha | *Saussurea lappa* C.B. Clarke | Root | 1part |
| 10. | Daruharidra | *Berberis aristata* D.C. | Root | 1part |
| 11. | Amalaki | *Embelica officinalis* Gaertn. | Fruit | 1part |
| 12. | Haritaki | *Terminalia chebula* Retz. | Fruit | 1part |
| 13. | Bibhitaki | *Terminalia belerica* Roxb. | Fruit | 1part |
| 14. | Danti | *Baliospurmum montanum* Muell. | Root | 1part |
| 15. | Chavya | *Piper chaba* Hunter | Root | 1part |
| 16. | Indrayava | *Holarrena antidysentrica* Wall. | Seed | 1part |
| 17. | Pippali | *Piper longum* Linn. | Fruit | 1part |
| 18. | Pippalimula | *Piper longum* Linn. | Root | 1part |
| 19. | Musta | *Cyperus rotundus* | Rhizome | 1part |
| 20. | Haridra | *Curcuma longa* Linn. | Rhizome | 1part |
| 21. | Shuddha Mandura | Red oxide of Iron, $Fe_2O_3$, $H_2O$ | | 40 part |
| 22. | Gomutra | Cow's Urine | | 160 part |

**Table 2: Organoleptic Features of Punarnavadi Mandura**

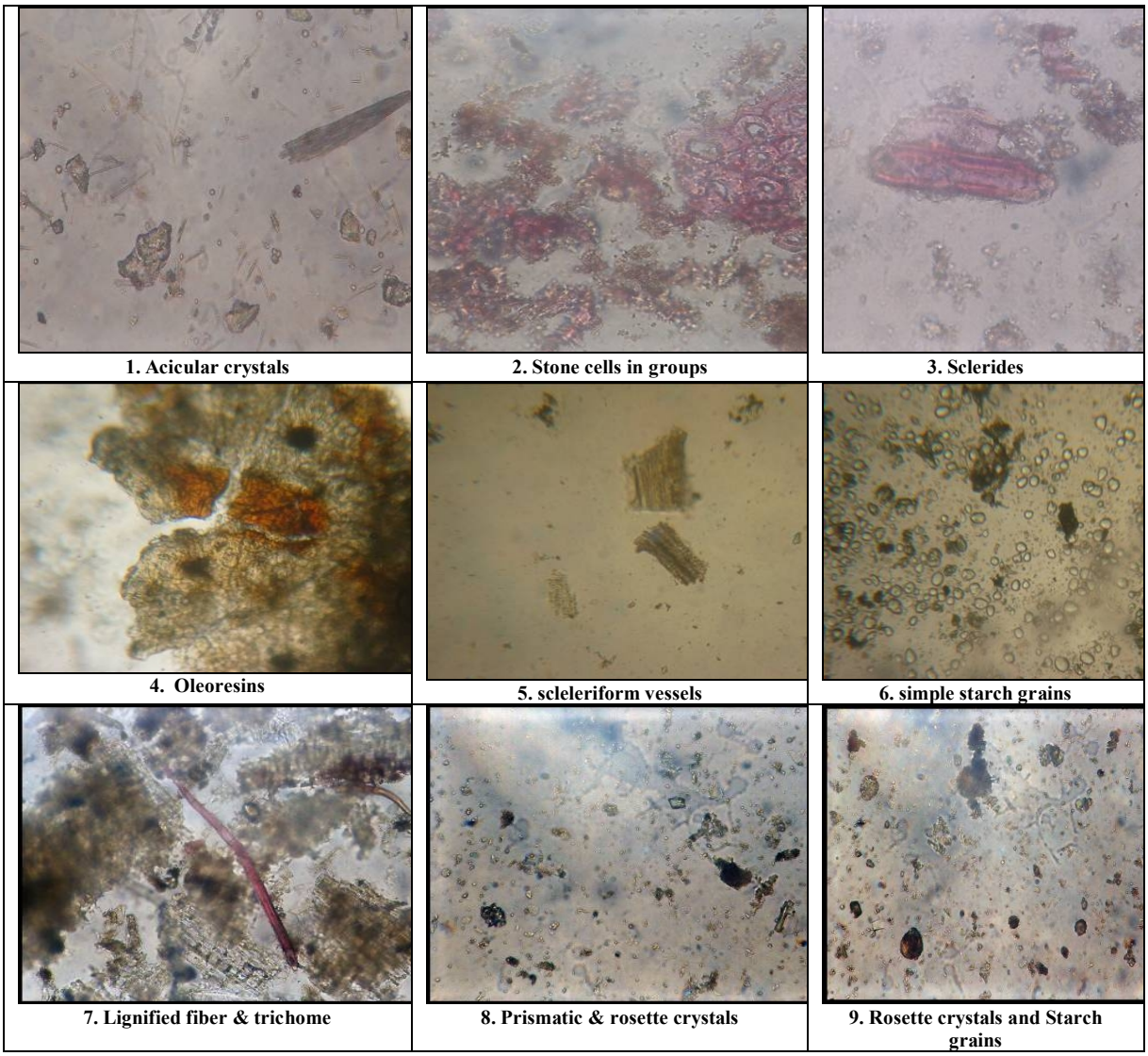
| Sr. No. | Characters | Observed |
|---|---|---|
| 1 | Texture | Rough |
| 2 | Colour | Brownish |
| 3 | Odour | Pungent (mild) |
| 4 | Taste | Astringent- Bitter |
| 5 | Consistency | Solid |

**Table 3: Physico-chemical Parameters of Punarnavadi Mandura**

| Sr. No. | Parameters | Punarnavadi Mandura Vati |
|---|---|---|
| 1 | **Uniformity of Tab.** | |
| | A- Average weight | 718 mg |
| | B- Highest weight | 885 mg |
| | C- Lowest weight | 654 mg |
| 2 | Tab hardness | 0.62 kg/cm$^2$ |
| 3 | Disintegration Time | 1 min. |
| 4 | Loss of drying at 110$^0$ C | 1.37 % w/w |
| 5 | Ash value | 71.12 % w/w |
| 6 | Water Soluble Extract | 13.09 % w/w |
| 7 | Methanol Soluble Extract | 6.37 % w/w |
| 8 | Ph (10% aqua solution) | 7.12 |
| 9 | Iron Content- as $Fe_2O_3$ | 49.15 %w/w |

**Table 4: Chromatographic Fingerprinting of Punarnavadi Mandura Vati MEOH Extracts on Silica Gel Gf 60 Plates**

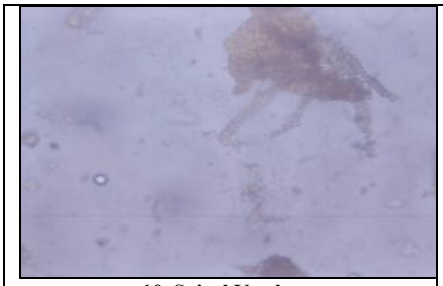
| solvent system | Short UV Radiation (254 nm) | | Long UV Radiation (366 nm) | | After derivetization Anisaldehyde: $H_2SO_4$ (0.5:5 v/v) followed by 105$^0$ C in oven | |
|---|---|---|---|---|---|---|
| | No of spot separated | $R_f$ | No of spot separated | $R_f$ | No of spot separated | $R_f$ |
| Toulene:Ethyl Acetate:AceticAcid (5:4.5:0.5 v/v) | 5 | 0.30, .66, 0.70,0.76, 0.85 | 2 | 0.31, 0.69 | 5 | 0.30, 0.66, 0.70, 0.76, 0.85 |



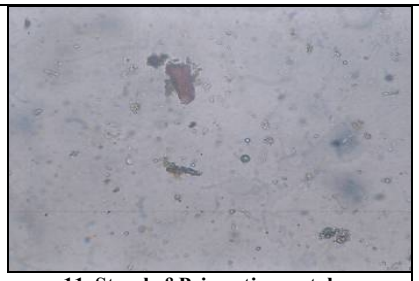
**1. Acicular crystals** | **2. Stone cells in groups** | **3. Sclerides**

**4. Oleoresins** | **5. scleleriform vessels** | **6. simple starch grains**

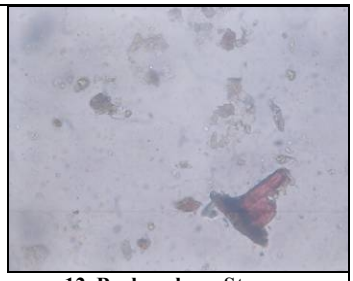
**7. Lignified fiber & trichome** | **8. Prismatic & rosette crystals** | **9. Rosette crystals and Starch grains**



**10. Spiral Vessles**



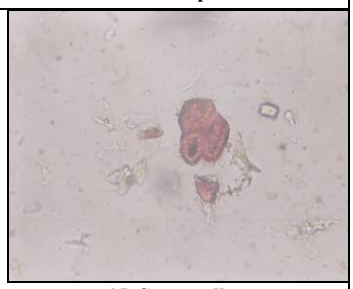
**11. Starch &Prismatic crystals**



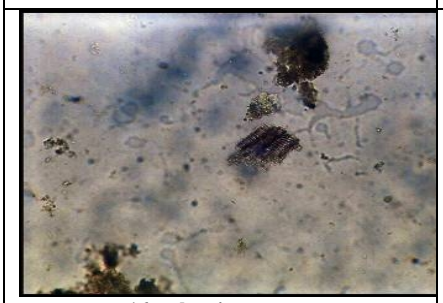
**12. Beaker shape Stone**

**13. Parenchyma**

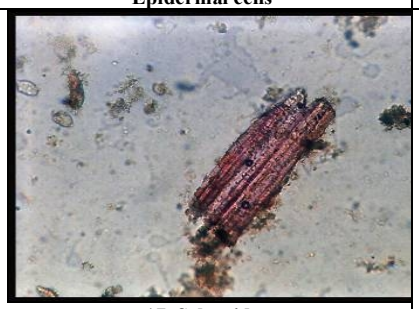
**14. Starch grain, prismatic crystals & Epidermal cells**



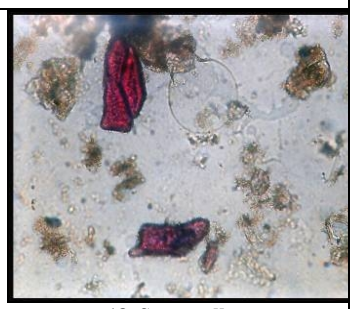
**15. Stone cells**



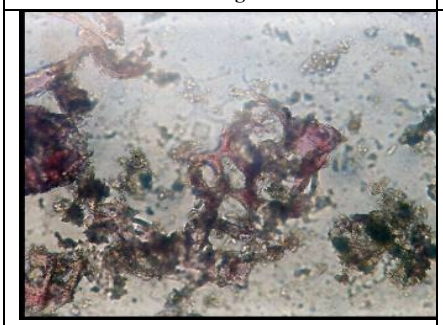
**16. colouring matter**



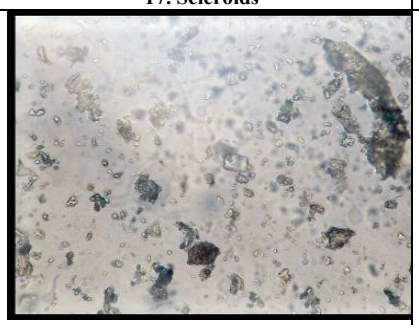
**17. Scleroids**



**18. Stone cells**

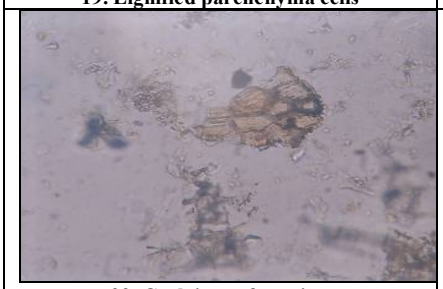


**19. Lignified parenchyma cells**



**20. Prismatic crystals**

**21. Stone cells**



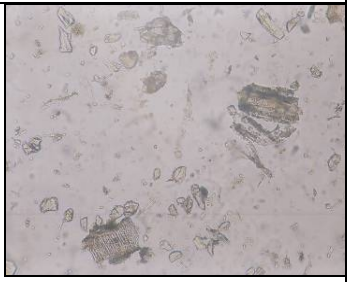
**22. Cork in surface view**

**23. Scleleriform vessels**

**24. Stone cells**

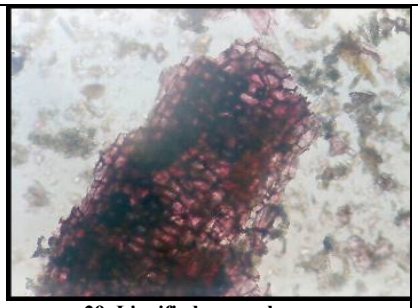
**25. cork in tangantial view**

**26. fiber with prismatic crystles**

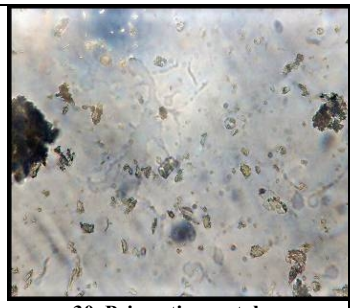


**27. Pitted vesseles**

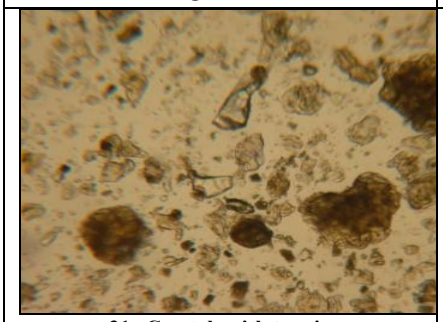
**28. Lignified fiber**



**29. Lignified parenchyma**

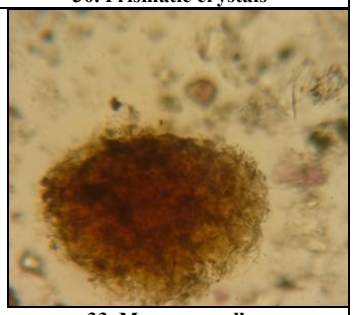


**30. Prismatic crystals**



**31. Crystals with tannin**

**32. Scleroids**

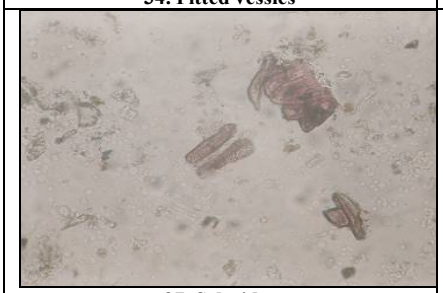


**33. Mesocarp cells**

**34. Pitted vessles**

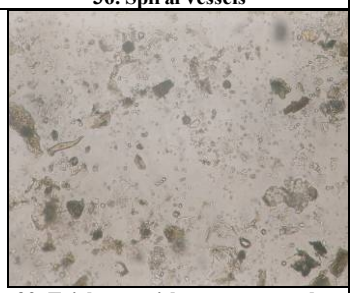
**35. Scleroids**

**36. Spiral vessels**

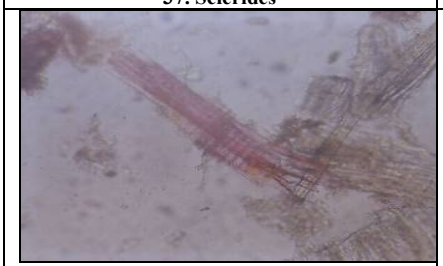


**37. Sclerides**

**38. Stone cells**

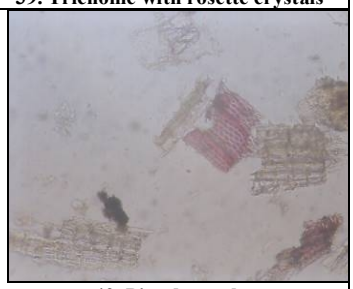


**39. Trichome with rosette crystals**

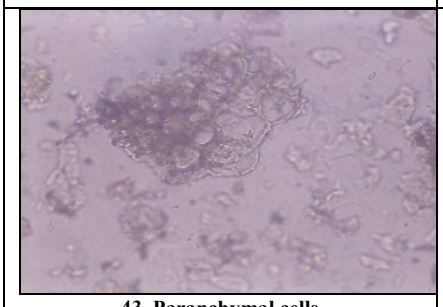


**40. fibers through medullary rays**

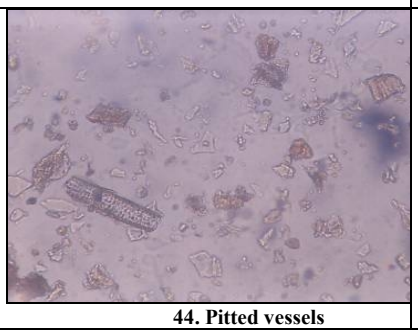
**41. Lignified fiber with cortical cells**



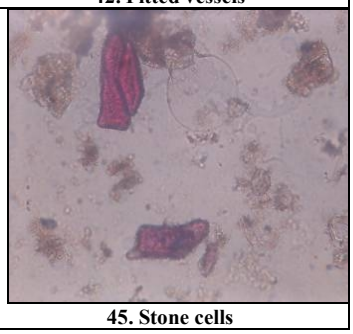
**42. Pitted vessels**



**43. Paranchymal cells**



**44. Pitted vessels**



**45. Stone cells**

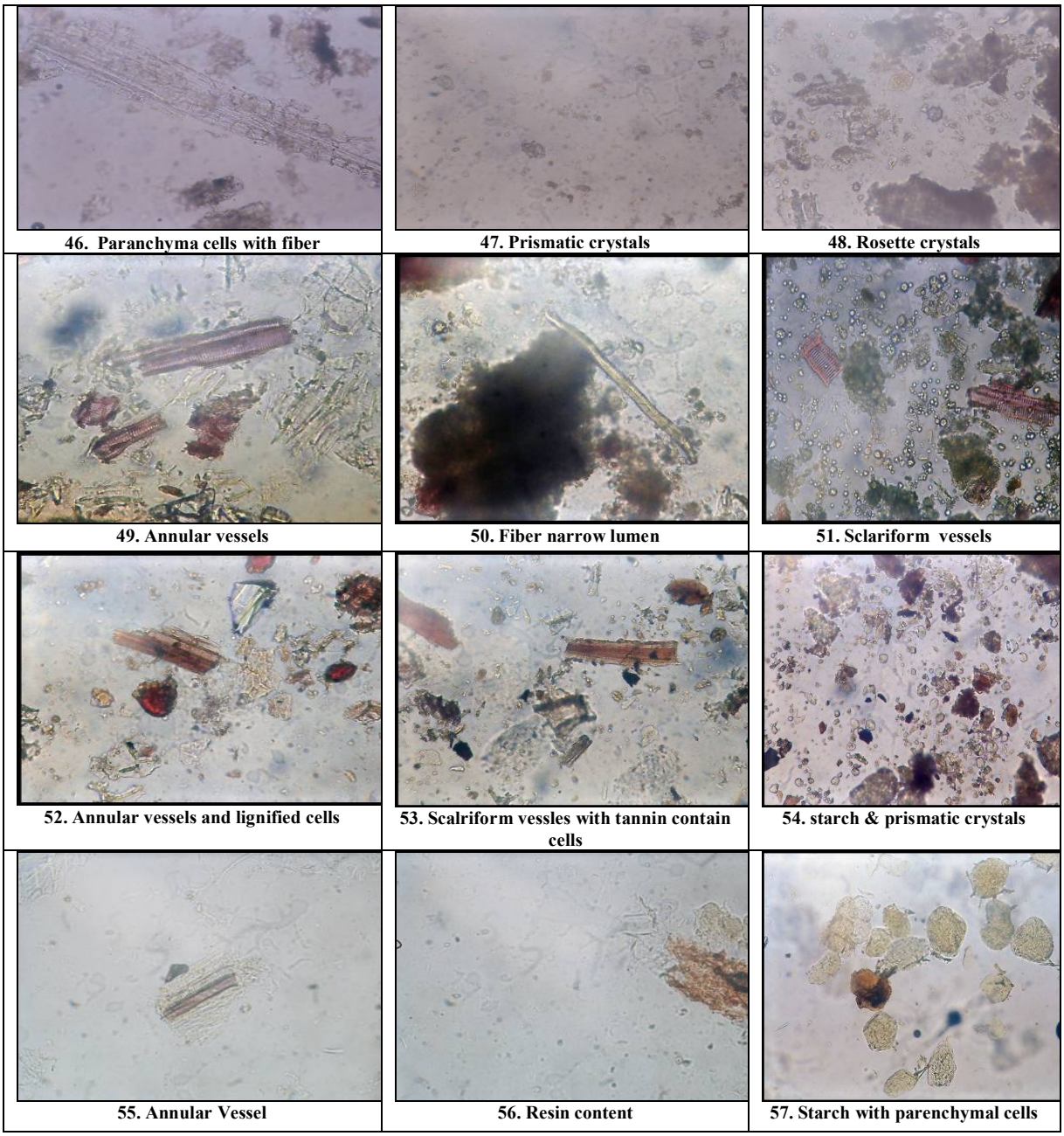



**Figure 1: Microphotographs of Punarnavadi ManduraVati**

## RESULTS AND DISCUSSION

### Pharmacognostical study

### Organoleptic evaluation

Punarnavadi Mandura Vati was light radish brown in color, Gomutra gandhi, Astringent- Bitter in taste and solid consistency with rough surface. Details are tabulated in table 2.

### Microscopic evaluation

Diagnostic characters of Punarnavadi Mandura vati were Acicular crystals, sclerides, stone cells in groups (Punarnava), oleoresins, simple starch grains, scleleriform vessels (Shunti), Lignified fiber & trichome, rosette crystals and starch grains, prismatic & rosette crystals (Trivrita), Spiral Vessles, beaker shape Stonecell, starch & prismatic crystals (Maricha), Parenchyma, prismatic crystals & epidermal cells (Pippali), colouring matter, scleroids (Vidanga), Lignified parenchyma cells (Devdaru), Cork in surface view (Chitraka), cork in tangantial view, fiber with prismatic crystles (Kushtha), Crystals with tannin, mesocarp cells of scleroids (Amlaki), pitted vessels(Haritaki), Scleroids, stone cells, rostte crystel with trichome (Bibhitaki), fibers through medullary rays, Pitted vessels, lignified fiber with cortical cells (Dhanti), Paranchymal cells, stone cells, pitted vessels (Chavya), Parenchyma cells with fiber, rosette crystals, prismatic crystals(Indrayava), Annular vessels, sclariform vessels, fiber narrow lumen (Pippalimoola). Annular vessels and lignified cells,starch & prismatic crystals, scalriform vessles with tannin contain cells (Musta), Annular Vessel, starch with parenchymal cells, resin content (Haridra). Microphotographs are shown in Figure 1-1 to 57.

**Physico - chemical Parameters**

Physico- chemical Parameters were within the normal range. The water soluble extract and methanol soluble extract values were found to be 13.09 % w/w and 6.37 % w/w respectively (Table 3).

**High Performance Thin Layer Chromatography Study**

Densitometric scanning of the HPTLC pattern showed 5 spots corresponding to $hR_f$ values 0.30, 0.66, 0.70, 0.76, 0.85 in short wave UV 254 nm and 2 spots corresponding to $hR_f$ values 0.31, 0.69 obtained in long wave UV 366 nm (Table 4).

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