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## Research Article

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## A Preliminary Pharmacognostical and Physico Chemical Assay of Pippalikhanda Granules

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### ABSTRACT

During an ethnobotanical survey conducted among the Zafimaniry clan in the Amoron'i Mania region of Madagascar, 164 medicinal plants species from 138 genera and 73 families are recorded to be used by local people to treat 65 different types of human diseases. The most prescribed species belong to Asteraceae (30 species), Fabaceae (8 species), Rubiaceae (7 species) Poaceae (6 species) and Solanaceae (7 species) botanical families. *Helichrysum* was the most cited genus with 6 species. The main pathologies treated with medicinal plants are abdominal colic (10.5%) using 25 species, cough (7.2%, 23 species), intestinal parasites (6.3%, 20 species) and diarrhea (5.8%, 25 species). The vernacular names and the recipes of used plants are also reported. Data are collected by interviewing 191 informants aged between 16 and 100 using standardized questionnaires. The present study shows that the Zafimaniry people use a large number of medicinal plants for their primary health care. Some of the recorded plants are new in the Malagasy ethnopharmacopoeia.

**Keywords:** Medicinal plants, Ethnomedicine, Zafimaniry clan, Madagascar.

### INTRODUCTION

In present days *Amlapitta* is found to be very widespread disease socially. Due to irregular dietary habits, faulty diets, hectic life style etc. prevalence of this disease has increased. *Acharya* has incorporated *Amlapitta* in *Viruddhaharajanya Vyadhi*. Pippalikhanda is indicated in the management of *Amlapitta* i.e. Hyperacidity in one of the certified text book of Ayurveda i.e. *Bhaishajya Ratnavali Amlapitta prakaran* [1]. It contains

*Pippali, Shatawari, Twak, Ela, Tejpatra, Musta, Dhanyaka, Shunthi, Vanshlochana, Svetajiraka, Krsnajoraka, Haritaki, Amalaki, Khadirasara, Maricha, Sharkara, Godugdha, Goghrita, Kshaudra.*

All the Ingredients of Pippalikhanda are having quality to control the acidity and to better the digestion. Significant result was showed with *Pippalikhanda* Granules in the management of *Amlapitta* i.e. Hyperacidity.

*Amlapitta* (Hyperacidity) is a common digestive problem recognized since ancient days. In "Bhaishajya Ratnavali" a separate chapter has been described for *Amlapitta* with all of its clinical entity in which there is description of *Pippalikhanda* for the treatment of *Amlapitta* which denotes all of its importance. There are two types of *Amlapitta* i.e. Urdhwaga *Amlapitta* and Adhoga *Amlapitta* [2], both of them has their different and unique characteristics, which is very difficult to treat. Hence in this present study, Pippalikhanda Granules is selected for the evaluation of its efficacy clinically in the treatment of *Amlapitta* i.e. Hyperacidity.

in *Avaleha & Khanda Kalpana* fungus formation is found to be a common problem as Jamnagar is found a coastal area, hence, to improve the palatability and increase life span, *Pippalikhanda* has been prepared in granular form. Proper recognition and standardization of this drug is necessary and each ingredient in this formulation has some specific physical and chemical characteristics that help in separating it from other nearly identical drugs. For the standardization of drug, physicochemical analysis by various parameters are needed. Hence, with the help of pharmacognosy the raw drugs were recognised and authenticated. Then with the help of standard operating procedure, granules were prepared and standardization of the finished product have been done by the Pharmacognostical and Physico-chemical analysis to avoid the controversy related with the ingredients present in Pippalikhanda.

## Aims & Objectives

- Authentication of raw drugs of *Pippalikhanda*.
- Authentication of final product of *Pippalikhanda*.
- Physico-chemical analysis of *Pippalikhanda* granules.

## MATERIALS AND METHODS

### ➤ Ingredients of Pippalikhanda Granules

The composition of *Pippalikhanda* along with the individual components with their proportion and amount is listed out in Table 1.

### ➤ Collection of Raw Drugs

Among the raw material of *Pippalikhanda* i.e. *Pippali*, *Shatawari*, *Twak*, *Ela*, *Tejpatra*, *Musta*, *Dhanyaka*, *Shunthi*, *Vanshlochana*, *Svetajiraka*, *Krsnajiraka*, *Haritaki*, *Amalaki*, *Khadirasara*, *Maricha*, *Sharkara*, *Godugdha*, *Goghrita*, *Kshaudra* all the drugs except *Goghrita*, *godugdha* and *Madhu* were collected from the Pharmacy, Gujarat Ayurved University Jamnagar. *Goghrita*, *godugdha* and *Madhu* were purchased from the market of Jamnagar.

**Recognition and Authentication of ingredients-** The ingredients were recognized and authenticated in the Pharmacognostic laboratory of IPGT & RA, GAU, Jamnagar. On the basis of organoleptic characteristic powdered microscopy and morphological characters identification of the individual drugs has been done. The API [3] is authenticated reference for this.

### Method of Preparation of the Pippalikhanda Granules

Formation of *Pippalikhanda* Granules has been done at Pharmacy of I.P.G.T. & R.A., GAU, Jamnagar. raw drugs of *Pippalikhanda* were first dried then powdered separately and through the sieve 85# in order to make fine powder and then this raw drug were mixed in specified quantity (Except *Pippali* and *Shatawari*) to make a homogeneous mixture.

First of all, *Pippali* churna was fried in *Goghrita* and the *shatawari* kwath was mixed in it and boil it until it became thick. Then warm milk was added in it and boiled again, when very few amount of milk was remained then and it became as thicker as *do taar chashni* then all the prakshep was added in it to make it and it was mixed properly then left it for few minute to make its temp. low then it was passed through sieve to make its granules and allowed it to cool when moisture was removed then it was filled in air tight packet [4].

## PHARMACOGNOSTICAL ANALYSIS

### Organoleptic Characteristic

Organoleptic characteristic of this sample is described in table 2 according to Pharmacognostical study.

### Powder Microscopy

Diagnostic microscopic characters of *Pippalikhanda* Granules are – Accicular crystal *Shatawari*, black debris of *Maricha*, bottle neck shaped cells of *Pippali*, bottle neck stone cell *Maricha*, Cork cells in surface of *Twak*, Endosperm cells of *Krishnajeera*, Epidermal cells of *Tejpatra*, lignified scleroid of *Amalaki*, lignified fibre of *Musta*, mesocarp cells *Haritaki*, Mesocarp with tannin of *Pippali*, oil globule of *Krishnajeera*, oil globules of *Goghrita*, Olio-resine with starchgrains of *Shunthi*, Parenchyma cells with starch grains of *Musta*, perisperm cells of *ela*, Schelerides with stain of *Twak*, scleride of *Haritaki*, simple fibers of *Krishna Jeera*, starch grains with hylem along with prismatic crystals of *Khadir sara*, Stomata of *Dhanyak*, stone cells of *ela*, Trichome of *Tejpatra*, Vittae cells of *Jeera*, wavy parenchyma cells of *Dhanyak*.(plate described as below)

All the above-mentioned microscopic characteristics were same as the standard individual character as mentioned in API.

## Physico-Chemical Analysis

### Material for Physico - Chemical study of finished product

With the help of qualitative and quantitative parameters *Pippalikhanda* Granules were analyzed at Pharmaceutical Laboratory of I. P. G.T & R. A., Gujarat Ayurved University, Jamnagar.

For physico-chemical parameters like Loss on drying, Total Ash Value, Sugar estimation, pH value, Alcohol soluble and water-soluble extractive values the granules were studied. As we know that Presence of more moisture is responsible for destroying the mater and thus can create problem in the preservation of drug that's why loss on drying is one of the very important parameters. Since, the sample was in granular form, and granules must have sugar in that so the estimation of sugar was also a very important parameter. The results are showed in table 3.

In small quantity of distilled water *Pippalikhanda* Granules were dissolved filtered through filter paper, and then filtered and dried and finally observed under microscope with and without stain.

### Microscopic preparation of methanol extract

The amount of the granule which was taken for the examination was 5 gm with 100 ml of alcohol, it is kept for 24 hours, the filtrate which we found were evaporated in order to make it dry in a shallow dish and again concentrated until we got the volume that we required

### Thin Layer Chromatography

For identification of monographs on all medicinal plants, TLC is mentioned as a primary tool. spotting of the TLC plate (Silica gel G Precoated plates) was done with the alkaloid fraction. And then this spotted TLC was kept under the solvent systems (Toluene (7 ml), Glacial acetic acid (0.5 ml), Ethyl acetate (2 ml), Formic Acid (0.5 ml)) separately; then it was viewed in the long wave UV light at 366 nm or Short-wave UV light at 254 nm. This process was Repeated again and again in order to develop proper solvent system and then this solvent system was finalized which was prepared by this method.

### High Performance Thin Layer Chromatography

First of all, Methanol extract of *pippalikhanda* was prepared then it was spotted on pre coated silica gel GF 60254 aluminium plate as 5mm bands, which was 5mm apart from each other and 1cm from the edge of the plates, with the help of a Camag Linomate V sample applicator fitted in a 100 µL Hamilton syringe. Glacial acetic acid, Ethyl acetate (2 ml), Toluene (7 ml), were used and micro photographs was taken under corl zeiss binocular microscope which was attached with camera as shown in table no. 4.

## DISCUSSION

Organoleptic and Microscopic evaluation of fine powdered ingredients was done as per the guidelines of Ayurvedic pharmacopoeia of India at Pharmacognostical laboratory, I.P.G.T & R.A, GAU, Jamnagar.

After progress, Densitometric scanning was performed with the help of Camag T.L.C. scanner III in reflectance concentration mode at 254 nm and 366 nm under control of win CATS software (V 1.2.1 Camag). The slit dimensions were found to be 6 mm x 0.45 mm and the scanning speed was 20 mm s-1. Then Anisaldehyde Sulphuric acid sprayed on the plate which was followed by heating and then observed in day light. The results of HPTLC of Methanolic extract of *Pippalikhanda* Granules at 254 nm, 366 nm and in clear day light after spraying Anisaldehyde Sulphuric acid are shown in table 5. This drug was prepared and analysed for the treatment of *Amlapitta* so effort has been made to prepare a drug which has positive effect on the treatment of *Amlapitta*

and this formulation contain many individual drugs which directly or indirectly on the disease Amlapitta i.e. Hyperacidity.

Pharmacognostical study showed that organoleptic feature of the prepared drug was greenish brown in colour, offensive smell in odor, sweetish followed by pungent in taste and granular in touch.

Microscopic study showed that presence of accicular crystal Shatavari, black debris of maricha, bottle neck stone cell maricha, Cork cells in surface of Twak, Bottle neck shaped stone cells of Pippali, Endosperm cells of Krishnajeera, Epidermal cells of Tejpatra, lig. sclerid of amalaki, lignified fibre of Musta, mesocarp cells haritaki, Mesocarp with tannin of Pippali, oil globule og Krishnajeera, oil globules of Goghrihta, Olioesine with starchgrains of Shunti, Parenchyma cells with starch grains of Musta, perisperm cells of ela, Schelerides with stain of Twak, scleride of haritaki, simple fibers of Krishna jeera, starch grains with hylem along with prisamatic crystals of Khadir sara, Stomata of Dhanyak, stone cells of ela, Trichome of Tejpatra, Vittae cells of Jeera, wavy parenchyma cells of Dhanyak showed that all the ingredients were present in the formulation and also proved that the specificity of the formulation.

**CONCLUSION**

The prepared drug Pippalikhandha Granules was subjected to pharmacognostical, physico-chemical analysis in the laboratory of IPGT AND RA Jamnagar. All the details of pharmacognostical and pharmaceutical study was obtained with the help of scholars and teachers present there. The detail of Pippalikhnada granules are present only in Bhaisajya Ratnawali and Bhava Prakash. No any other description presents in any other Samhita and API, AFI etc. And no any work has been done on this drug before.

**Table 1:** Content of Pippalikhandha

S.N.	Drug	Latin Name	Part Used	Quantity
1.	Pippali	<i>Piper longum</i> Linn.	Fruit	1 pala
2.	Shatavari	<i>Asparagus racemosus</i> Willd.	Root	8 pala
3.	Twak	<i>Cinnamomum zeylanicum</i> Breyn.	Bark	2 masa
4.	Ela	<i>Elettaria cardamomum</i> Maton.	Fruit	2 masa
5.	Tejpatra	<i>Cinnamomum tamala</i> Breyn.	Patra	2 masa
6.	Musta	<i>Cyperus scariosus</i>	Root	2 masa
7.	Dhanyaka	<i>Coriandrum sativum</i> Linn.	Fruit	2 masa
8.	Shunthi	<i>Zingiber officinate</i> Roxb.	Rhizome	2 masa
9.	Vanshlochana	<i>Bambusa arundinacia</i>	Niryas	2 masa
10.	Svetajiraka	<i>Cuminum cyminum</i> Linn.	Fruit	2 masa
11.	Krsnajiraka	<i>Carum carvi</i> Linn.	Fruit	2 masa
12.	Haritaki	<i>Terminalia chebulla</i> Retz.	Fruit	2 masa
13.	Amalaki	<i>Ambalica officinalis</i> Gaertn.	Fruit	2 masa
14.	Khadirasara	<i>Acacia catechu</i> Linn.f.	Niryas	6 masa
15.	Maricha	<i>Piper nigrum</i> Linn.	Fruit	6 masa
15.	Sharkara	Sugar		16 pala
16.	Godugdha	Cow's milk	Milk	32 pala
17.	Goghrihta	Cow's Ghee	Ghee	6 pala
18.	Kshaudra	Honey	Honey	2 pala

**Table 2:** Organoleptic characteristics of Pippalikhandha granules

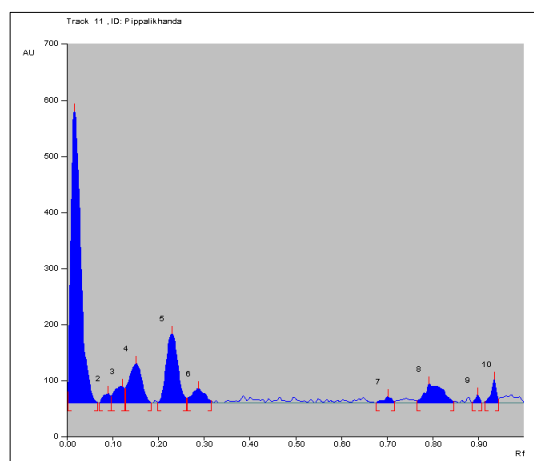
Sample	Parameters				
	Colour	Odour	Taste	Touch	Touch
Pippalikhandha	Greenish Brown	Offensive smell	Sweetish f/b Pungent	Granular	Grannular

**Table 3:** Physico – Chemical Parameters of Pippalikhandha Granules

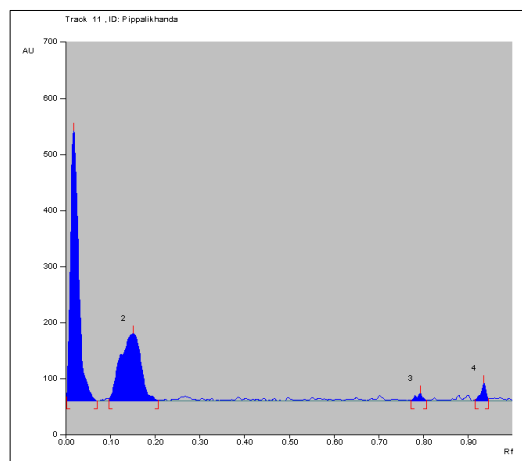
S. No.	Parameter	Pippalikhandha
1	Loss on drying	14.214% w/w
2	Ash value	5.234% w/w
3	Water soluble extractive	54.34% w/w
4	Metanol soluble extractive	36.34% w/w
5	pH value	5

**Table 4:** HPTLC Findings of Pippalikhandha Granules

S.N.	Visualization	No. of spots
1.	254 nm	10
2.	366 nm	4



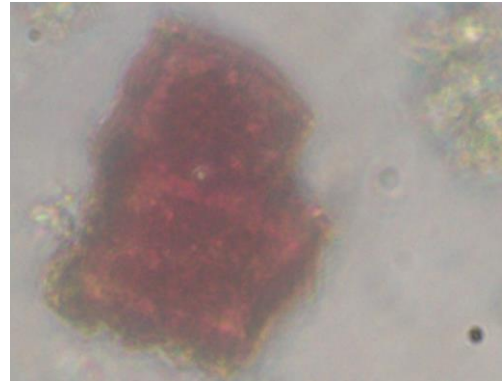
**Figure 1:** 254 nm Peak display (10 spots )



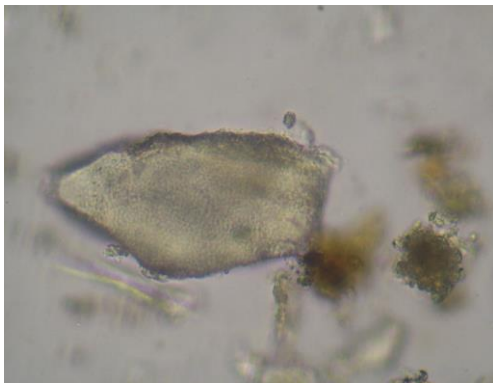
**Figure 2:** 366 nm Peak display ( 4 spots )



Accicular cry. shatavari



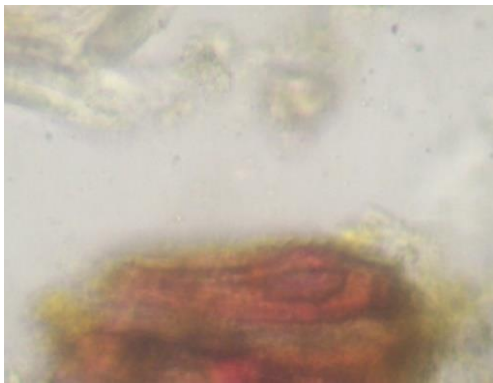
Bottle neck shaped stone cells of Pippali



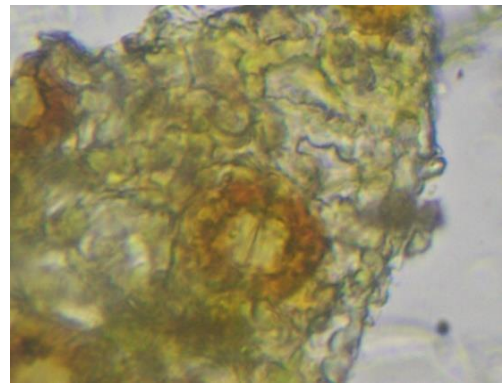
Black debris of maricha



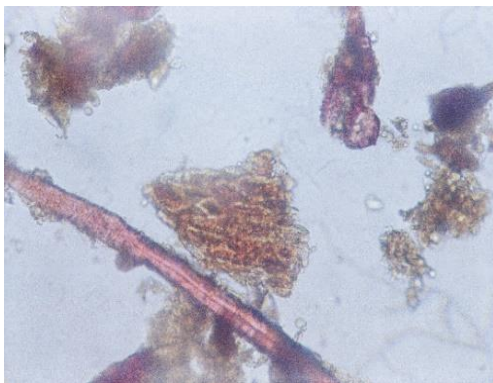
Endosperm cells of Krishnajeera



Bottle neck stone cell maricha



Epidermal cells of Tejpatra



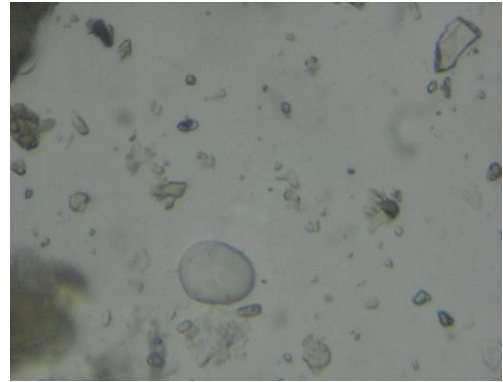
Cork cells in surface of Twak



Lig. sclerid of amalaka



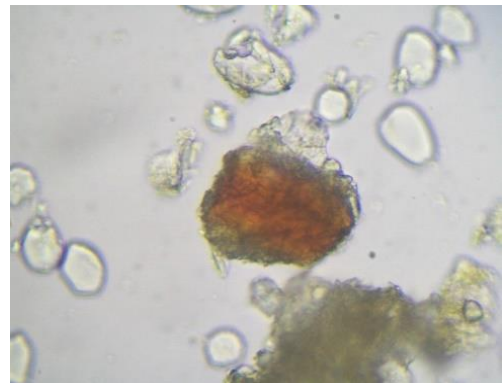
Lignified fibre of Musta



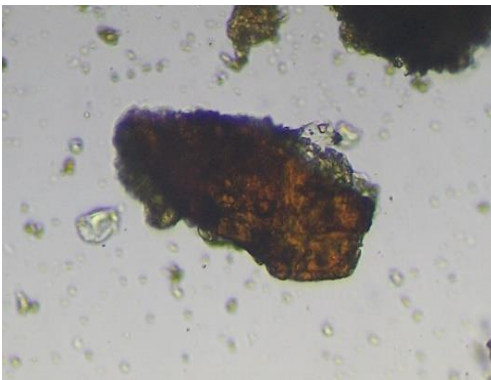
Oil globules of Gogrihta



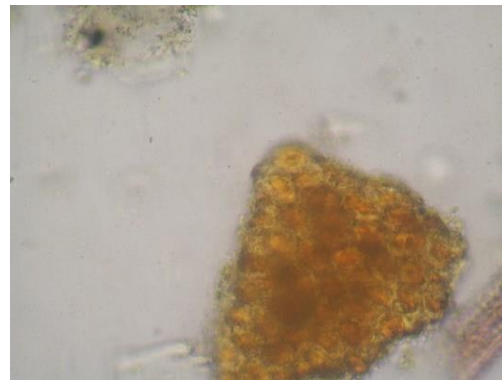
Mesocarp cells haritaki



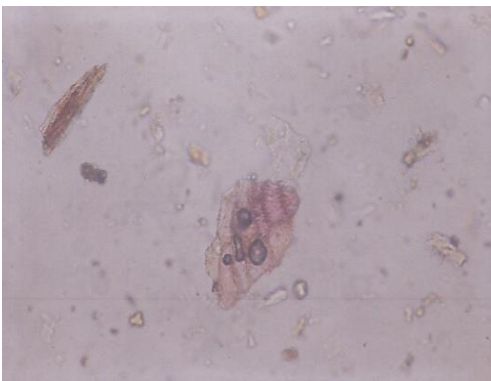
Olioresine with starchgrains of Shunti



Mesocarp with tannin of Pippali



Parenchyma cells with starch grains of Musta



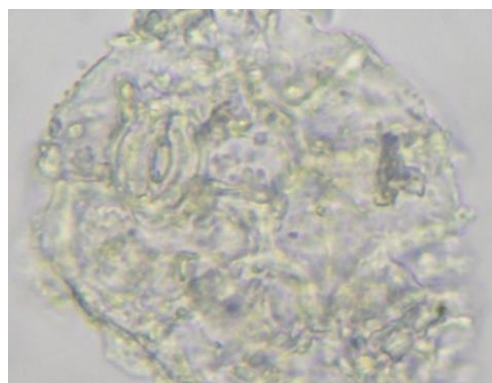
Oil globule og Krishnajeera



Perisperm cells of ela



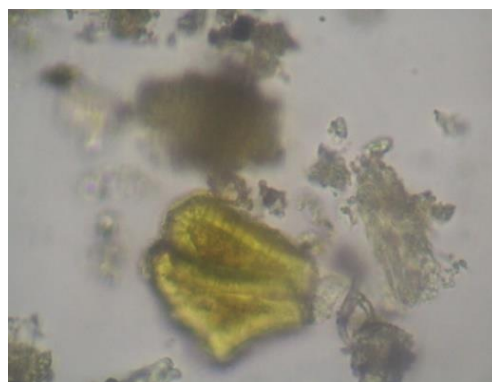
Schelerides with stain of Twak



Stomata of Dhanyak



Scleride of haritaki



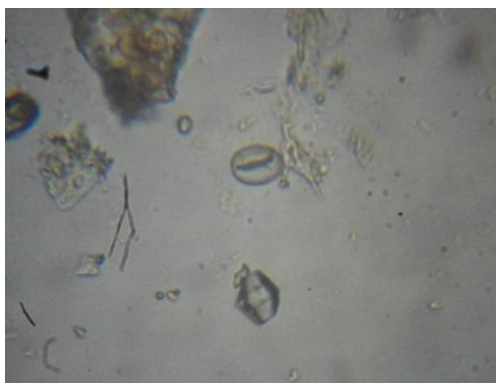
Stone cells of ela



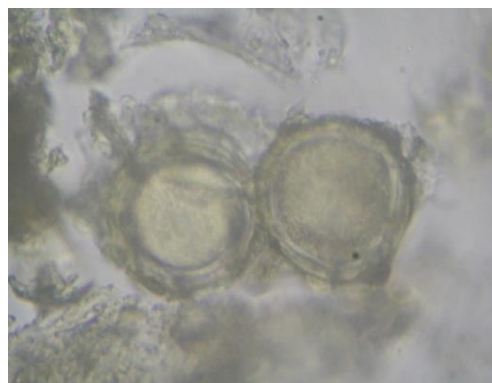
Simple fibers of Krishna jeera



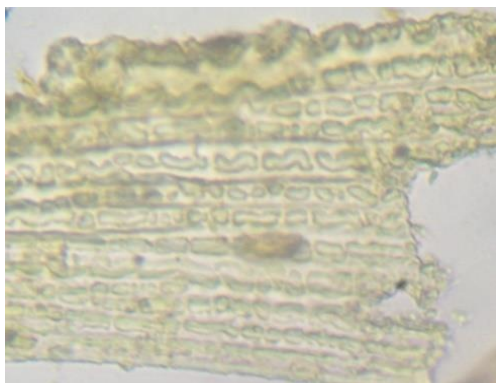
Trichome of Tejpatra



Starch grains with hylem along with prisamatic crystals of Khadir sara



Vittae cells of Jeera



Wavy parenchyma cells of Dhanyak

**Table 3:** Plates for Microphotographs of *Pippalikhanda*

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