International Journal of Ayurveda and Pharma Research

Research Article

PHARMACOGNOSTICAL AND PHYTO-CHEMICAL EVALUATION OF *PIPPALYADI YOGA*: A POLYHERBAL FORMULATION

Pandya Shachi Hemantkumar[1*], Kamini Dhiman[2], L. P. Dei[3], A. B. Thakar[4], Harisha C R[5]

*[1]P.G. Scholar, [2]Guide and Reader, [3]Co-guide, I/C HOD, Dept. Stree roga and Prasuti Tantra, Institute for Post Graduate Teaching & Research in Ayurveda, Jamnagar, Gujarat, India.

[4]Co-guide, HOD, Dept. Panchakarma, Institute for Post Graduate Teaching & Research in Ayurveda, Jamnagar, Gujarat, India.

[5]Head, Pharmacognocy lab, Institute for Post Graduate Teaching & Research in Ayurveda, Jamnagar, Gujarat, India.

ABSTRACT

Pippalyadi Yoga is *Churna Kalpana* described by *Acharya Chakrapani* in *Vandhyatva* (infertility). Ovulatory dysfunction is the prime cause of Infertility among the world, *Pippalyadi Yoga* is useful in patients especially having Anovulation which is known as *Abeejatva* in *Ayurveda*. So a new pharmaceutical preparation *Pippalyadi Yoga* in the form of *Churna* (powder) was tried to standardize which is economical in terms of time and machinery usage. Pharmacognostical and phyto-chemical observations revealed the specific characters of all active constituents used in the preparation. The present work was carried out to standardize the finished product of *Pippalyadi Yoga* to confirm its identity, quality and purity. The presence of stone cells, oil globules, olio resin cells, parenchymatous cells, oval & beaker shaped starch grains, pollen grains were the characteristic features observed in the microscopy of the prepared drug. Phyto-chemical analysis showed Loss on drying 10.07 % w/w, ash value 6.55 %w/w, water soluble extract 14 % w/w, methanol soluble 13.40 % w/w, particle consistency above 60 mesh 4.10 % w/w, between 60-85 mesh 9.20 % w/w, between 85-120 mesh 13.30 % w/w & below 120 mesh 73.37 % w/w & pH 5.0.

HPTLC of *Pippalyadi Yoga* is the preliminary quantitative analysis which shows 8 prominent spots at Rf. 0.09, 0.61, 0.67, 0.74, 0.80, 0.86, 0.91, 1.00 in UV 254 nm and 7 prominent spots at Rf. 0.06, 0.17, 0.63, 0.67, 0.75, 0.82, 0.88 in UV 366 nm. *Pippalyadi Yoga*, a polyherbal formulation of 4 ingredients was prepared and HPTLC finger print profile was developed and it can be considered pharmacopial standard of *Pippalyadi Yoga*.

KEY WORDS: *Pippalyadi yoga*, Standardization, Pharmacognocy, Phyto chemical analysis, HPTLC.

INTRODUCTION

From time immemorial, *Ayurveda* has been a proven science of life & an efficacious way of treatment of human diseases coupled with holistic approach in diagnosing diseases and thereafter providing an all-inclusive treatment of body and soul with a sole aim of achieving complete cure. It has got amazing tools of preventive as well as curative methods.[1] In nutshell, it is an age old tested method of diagnosis coupled with proven method of herbal medicinal treatment. The line of treatment prescribed is not only effective but is also quite relevant in the modern age. In the olden days, the modality of diagnosis and drugs prescribed by masters were indubitably accepted and religiously followed by disciples.

In modern era, however, there has been a metamorphosis of approach. The values have changed, needs have undergone unprecedented change and there has been need to prove

consistency of medicine in its effectiveness and there is also need to ensure that there is no adulteration in the medicines used to ensure quality control as prescribed by World Health Organization (WHO). In order to achieve this, an ideal fusion of pharmacology and pharmaceutic is unavoidable. This is more essential because WHO has emphasized the need to ensure quality control of medicinal plant products by using modern technique and applying suitable standards.

In the light of the above, a study was conducted of *Pippalyadi Yoga*[2] which is a *Churna Kalpana* and is described by *Acharya Chakrapani* in his famous book *Chakradatta* in the treatment of Infertility. As we are aware, infertility is a global phenomenon in modern age as well. During the course of study it was observed that about 15 % of couples experience difficulty in conceiving[3]. It also displayed that ovulatory cause is an important subset in infertility among women, accounting about 40 % cases which includes Anovulation[4]. There is need to find out substitute in *Ayurveda* to eliminate hazards caused by Modern medication. *Pippalyadi Yoga* is one of such substitute which can be a hope for patients affected by infertility. So a clinical study was conducted to find out the role of *Pippalyadi yoga* in infertility w.s.r. to anovulatory factor. As a part of study, there was a need to ascertain and establish effectiveness of *Pippalyadi Yoga* to treat the infertility. Before starting the clinical trial, there was need to predetermine the fact that there is no adulteration and to ensure that the active constituents are as that were used in preparation of drug.

Keeping the Ayurvedic ethos in centrality, a systematic study was conducted to standardize the finished product of *Pippalyadi Yoga* using Pharmacognostical and Phyto-Chemical parameters. The authenticity, quality and purity of herbal drugs are established by references given in Pharmacopoeia.

MATERIALS AND METHODS

Collection of raw drugs

The raw drugs for the study were procured from the Pharmacy of Gujarat Ayurved University, Jamnagar. The final product i.e. *Pippalyadi yoga* was prepared in the Pharmacy of Gujarat Ayurved University, Jamnagar. The ingredients and parts used in the formation of *Pippalyadi Yoga* are listed in Table 1.

Table 1: Ingredients of *Pippalyadi Yoga*

NO	DRUG NAME	LATIN NAME	PART	PART USED	FORM
1	*Pippali*	*Piper longum* Linn.	1 gm	Fruit	Powder
2	*Shrungver/Shunthi*	*Zingiber officinale* Roxb.	1gm	Dried Rhizome	Powder
3	*Maricha*	*Piper nigrum* Linn.	1 gm	Fruit	Powder
4	*Nagkesara*	*Ochrocarpus longilolius* Benth. & Hook.f.	1 gm	Stamen	Powder

PHARMACOGNOSTICAL EVALUATION

Dry Powder of the *Prayojyaanga* of drugs which was being used in preparation of *Pippalyadi Yoga* had been used for this study. The root and powder characters were identified with the help of Pharmacognosy laboratory, Institute for Post Graduate Teaching & Research in Ayurveda, Gujarat Ayurved University, Jamnagar, India.

Powder microscopy of the sample was done without stain and after staining with Phloroglucinol + HCL. Microphotographs were taken under Carl Zeus microscope attached with camera.

PHYTO-CHEMICAL ANALYSIS OF DRUG

Pippalyadi Yoga was analyzed by using qualitative and quantitative parameters at Pharmaceutical Chemistry Laboratory, Institute for Post Graduate Teaching & Research in Ayurveda, Gujarat Ayurved University, Jamnagar, India.

The following tests were carried out.

- **Loss On Drying (LOD) [5]**

  This test was conducted to find out the moisture content in the samples.

Procedure: About 1gm, accurately weighed, sample was taken in a previously dried and weighed dish and heated in a hot air oven at 1100 C till constant weight. It was cooled and the weight was noted. Difference between the weight was calculated and taken as the loss on drying. The loss on drying of the sample was expressed as % w/w.

- **Ash Value (AV) [6]**

This test was carried out to evaluate the ash content of the samples.

Procedure: About 1 gm, accurately weighed, sample was taken in a previously weighed and dried crucible. It was then subjected to incineration in a muffle furnace without placing the lid on the crucible, allowed to cool and again weighed. From the obtained residue, the percentage of total ash content in the sample was calculated.

- **Water Soluble Extractive (WSE)[7]**

This test was carried out to evaluate the water soluble principles of the samples.

Procedure: 5gm of sample was weighed accurately, 100 ml of distilled water was added to it and it was kept overnight. Next day, it was filtered. 20 ml of the filtrate was transferred to a dried and weighed evaporating dish. The solvent was evaporated on a water bath, dried till constant weight, cooled and weighed immediately. From the weight of the residue, the percentage of water soluble extractive was calculated and expressed as %w/w.

- **Methanol Soluble Extractive (MSE)[8]**

This test was carried out to evaluate the methanol soluble principles of the samples.

Procedure: Methanol soluble extractive was determined by following the same method as mentioned in WSE, but methanol instead of distilled water.

- **Particle consistency**

This test was carried out to evaluate the consistency of the sample which is in powder form.

Procedure: Sample was passed through sieves having different size of mesh.

- **pH: [9]**

This test was carried out to find out the acidity or alkalinity of the samples.

Procedure: To 10 gm of the sample, 100 ml of distilled water was added, stirred for 2 hours, filtered, and the pH of the filtrate was noted in a Systronics pH meter.

- **High Performance Thin Layer Chromatography (HPTLC)[10]**

High performance thin layer chromatography (HPTLC) is an invaluable quality assessment tool for the evaluation of botanical materials. It allows the analysis of a broad number of compounds both efficiently and cost effectively. Additionally, numerous samples can be run in a single analysis thereby dramatically reducing analytical time. With HPTLC, the same analysis can be viewed using different wavelengths of light thereby providing a more complete profile of the plant than is typically observed with more specific types of analyses.

RESULTS AND DISCUSSION

Organoleptic Parameters

The organoleptic characters of Ayurvedic drugs are evaluating the qualities of preparation by color, touch, fineness, taste, odor, etc. were noted through Jyanendriya (sense organs) and it is providing the idea about the quality of different formulations without using chemical tests.

The present drug is having the Yellowish brown colour, pungent taste and astringent odour. The final product was made of fine powder form. Organoleptic Parameters of the formulation are mentioned in Table 2.

Table 2: Organoleptic Properties of *Pippalyadi Yoga*

Rupa (colour)	Yellowish brown
Rasa (Taste)	Pungent
Gandha (Odour)	Astringent
Sparsha (Consistency in Touch)	Fine Powder

This characters corresponds to the all active ingredients among which most of have *Katu Rasa* (pungent/hot).

Pharmacognostical evaluation

Diagnostic characters of finished product under the microscope were seen and presence of all ingredients showed their different characters.

- Stone cells, Tennin content, Fragment of pitted vessels & oil globule were seen.

Which are suggestive of *Pippali* shown in figures 7, 8, 9 and 10.

- Fragment of annular vessels, oleoresin content, parenchyma cells with starch grain, fibres were observed in the sample which are suggestive of *Shunthi* shown in figures 11, 12, 13, 14 and 15.
- Starch grain, beaker shaped stone cells, black debris along with parenchyma cells of suggests presence of *Maricha* shown in figures 16, 17 and 18.
- Golden yellow pollen grains having no protuberances were seen which are suggestive of one of the type of *Nagkesara-Sura punnaga* shown in figures 19 and 20.

PHYSICO-CHEMICAL PARAMETERS

Pippalyadi Yoga was evaluated for various physico-chemical parameters. The results are shown in table 3.

Table 3: Physico-Chemical Parameters of *Pippalyadi Yoga*

S.NO	TEST	RESULT
1.	Loss On Drying	10.07% w/w
2.	Ash Value	6.55% w/w
3.	Water Soluble Extract	14% w/w
4.	Methanol Soluble	13.40% w/w
5.	**PARTICLE CONSISTENCY**	
	A. Above 60 mesh	4.10% w/w
	B. Between 60-85 mesh	9.20% w/w
	C. Between 85-120 mesh	13.30% w/w
	D. Below 120 mesh	73.37% w/w
6.	pH	5.0

Loss on drying method is applied to determine the amount of water, all or a part of water for crystallization, or volatile matter in the sample. Loss on drying of test drug is 10.07 % w/w. Total ashes are designed to measure the total amount of material remaining after ignition. It includes both physiological (which is derived from the plant tissue itself) and non-physiological ash (residue of the extraneous matter etc. adhering to the plant substance) Ash value of powder is 6.55% w/w. Water soluble extract and alcohol soluble extract is 14% w/w & 13.40 % w/w respectively. Particle consistency is the test which defines fineness of powder. The results are shown in table. pH is the measure of acidity or basicity of a solution. In the present sample pH was detected by using pH indicator paper and Table 3 showing the acidic nature of the solution.

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

Thin layer chromatography is the most common form of chromatographic method used by Ayurvedic research workers to detect the number of compounds present in a product. It also helps to determine the purity of the sample. Identity of a compound is also possible by comparing it with the Rf value of a known compound.

Results are tabulated in Table-4. Chromatogram showed 8 prominent spots at Rf. 0.09, 0.61, 0.67, 0.74, 0.80, 0.86 0.91, 1.00 in UV254nm and 7 prominent spots at Rf. 0.06, 0.17, 0.63, 0.67, 0.75, 0.82, 0.88 in UV 366 nm. Densitometry at both wave length as well 3D view are also shown in pictures (figures 1, 2, 3, 4, 5, 6).

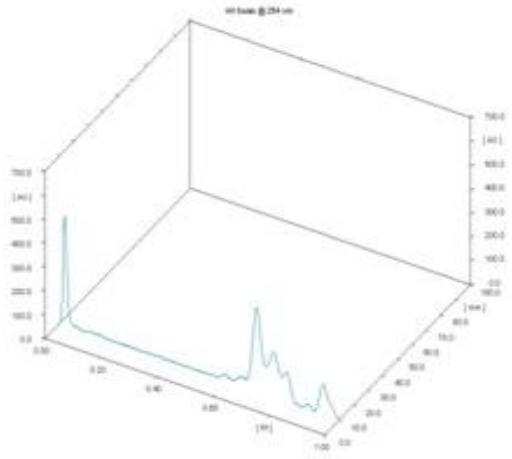

Fig 1: 254 nm 3d

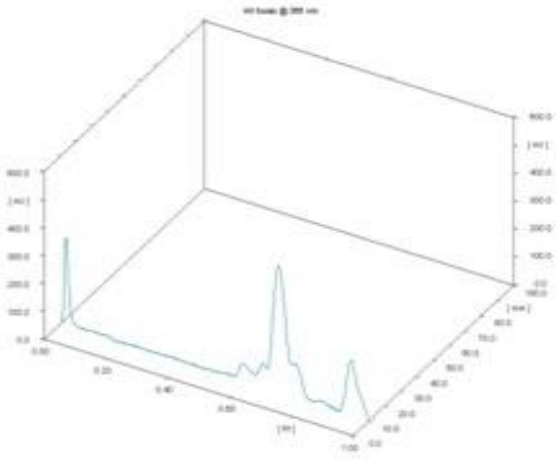

Fig 2: 366nm 3d

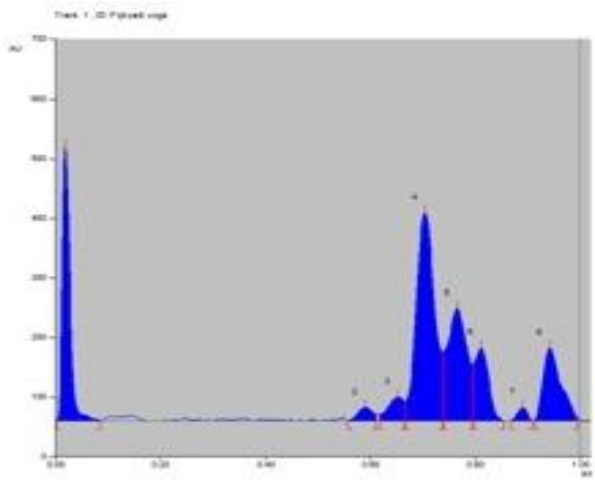

Fig 3: 254nm peak display

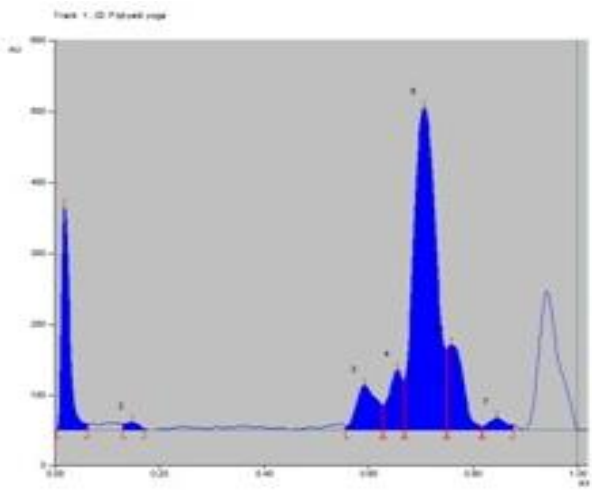

Fig 4: 366nm peak display

Fig 5: 254 nm

Fig 6: 366 nm

Table 4: HPTLC of *Pippalyadi Yoga*

Wave length	No. of spots	Rf value
UV-254 nm	8	0.09, 0.61, 0.67, 0.74, 0.80, 0.86, 0.91, 1.00
UV-366 nm	7	0.06, 0.17, 0.63, 0.67, 0.75, 0.82, 0.88

CONCLUSION

Pharmacognostical and phyto-chemical evaluation of *Pippalyadi Yoga* illustrated the specific characters of all ingredients which were used in the preparation.

The pharmacognostical and phyto-chemical analysis of *Pippalyadi Yoga* provides substantial information for the proper identification, authentication, and scientific evaluation of the final product/drug. On the basis of observations made and results of studies, this study may be beneficial for future researchers and can be used as a reference standard in the further quality control researches.

ACKNOWLEDGEMENT

The author gratefully acknowledges the valuable guidance provided by Dr. Kamini Dhiman (Guide), M.D(Ayu), Ph.D, the then Reader, Dept. Stree roga and Prasuti tantra at I.P.G.T. & R.A, Jamnagar, Gujarat, India & Dr. Harisha CR, Head, Pharmacognocy lab, IPGT & RA, Jamnagar, Gujarat, India; V.J.Shukla , Head, Pharmaceutical lab, I.P.G.T. & R.A., Jamnagar, Gujarat, India , Dr. L.P.Dei (Co-guide), I/C HOD, Dept. Stree roga & Prasuti tantra, I.P.G.T.&R.A, Jamnagar, Gujarat, Dr.A.B.Thakar, (Co-guide), HOD, Dept. Panchakarma, I.P.G.T.&R.A., Jamnagar, Gujarat.

REFERENCES

1. Agnivesha-Charaka Samhita with Ayurveda deepikavyakhya, by Vaidya Jadavji Trikamji Acharya, ed. Chaukhambha, Varanasi,2011, Sutra sthana, Ch-30/26;187
2. Chakrapani- Chakradatta by Jagdishvar Prasad Tripathi, Chaukhambha Sanskrut Series(1983)62/27;509
3. NICE-Fertility guidance-Feb 2004.www.nice.org.uk
4. Clinical Gynecologic Endocrinology and Infertility; 7th edition, by Leon Speroff et.al; published by Jaypee Brothers, 2005. Part IV; Chapter 27, page no. 1028.
5. The Ayurvedic Pharmacopea of India, 2007, 1st edition, Govt. of India, Part 2, Volume 1;appendix 2; 14
6. The Ayurvedic Pharmacopea of India, 2007, 1st edition, Govt. of India, Part 2, Volume 1;appendix 2; 13
7. The Ayurvedic Pharmacopea of India, 2007, 1st edition, Govt. of India, Part 2, Volume 1;appendix 2, 14
8. The Ayurvedic Pharmacopea of India, 2007, 1st edition, Govt. of India, Part 2, Volume 1;appendix 2; 14
9. The Ayurvedic Pharmacopea of India, 2007, 1st edition, Govt. of India, Part 2, Volume 1appendix 3; pg 65
10. Anonymous,1999,Planner Chromatography, Modern Thin layer Chromatography, Switzerland, pg 2-16

Cite this article as:
Pandya Shachi Hemantkumar, Kamini Dhiman, L. P. Dei, A. B. Thakar, Harisha C R. Pharmacognostical and Phyto-Chemical Evaluation of Pipalyadi Yoga: A Polyherbal Formulation. Int. J. Ayur. Pharma Research 2014; 2 (1): 116-123.
Source of support: Nil, Conflict of interest: None Declared

***Address for correspondence**
Dr. Pandya Shachi Hemantkumar
Practising Consultant
Plot No: 402/B
Opposite Anand Vihar
Prabhudas Talav
Bhavnagar-364001, Gujarat, India
Phone: +919687727988
Email: dr.shachipandya@yahoo.in

PHOTOGRAPHS OF MICROSCOPIC FEATURES INGREDIENTS OF *PIPPALYADI YOGA*

Fig 7: Stone cells of of *Pippali*

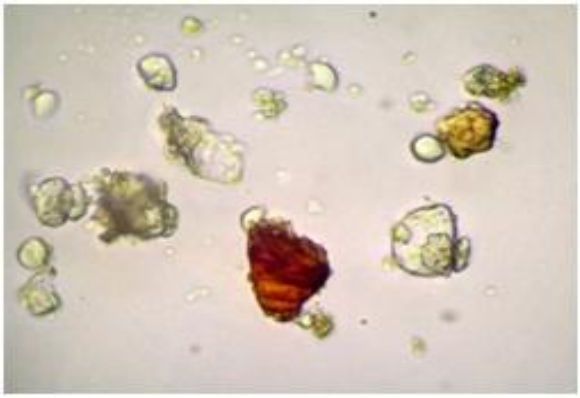

Fig 8: Tennin content of *Pippali*

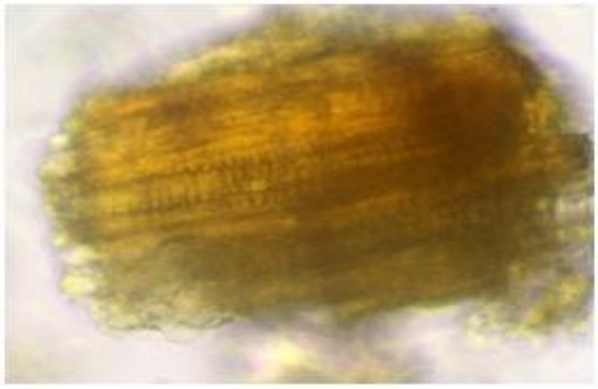

Fig 9: Fragments of pitted vessels of *Pippali*

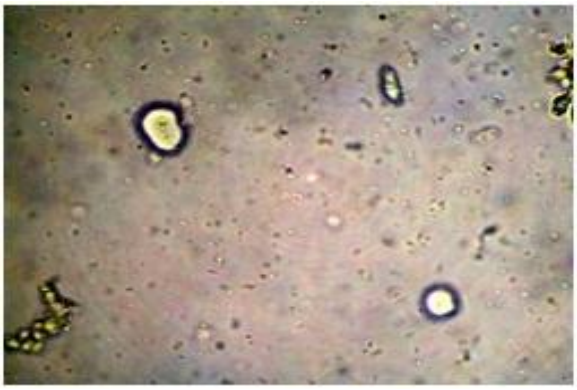

Fig 10: Oil globules of *Pippali*

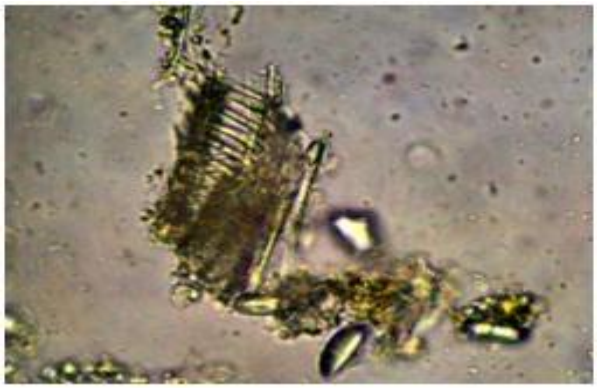

Fig 11: Fragments of annular vessels of *Shunthi*

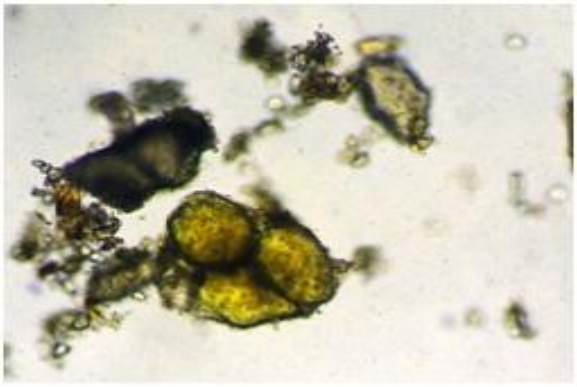

Fig 12: Oleoresin content of *Shunthi*

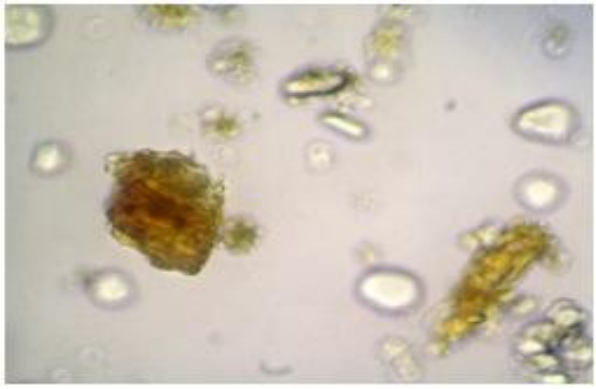

Fig 13: Oleoresin content of *Shunthi*

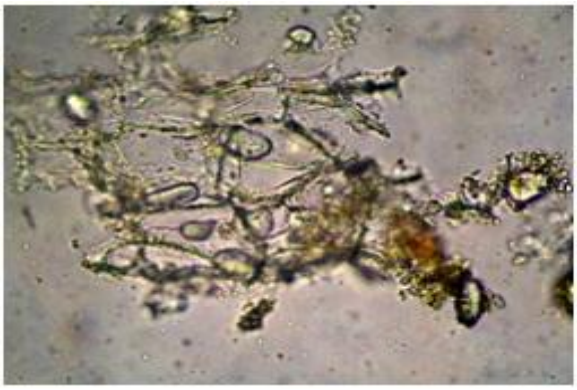

Fig 14: Parenchyma cells with starch grain of *Shunthi*

Fig 15: Fibres of *Shunthi*

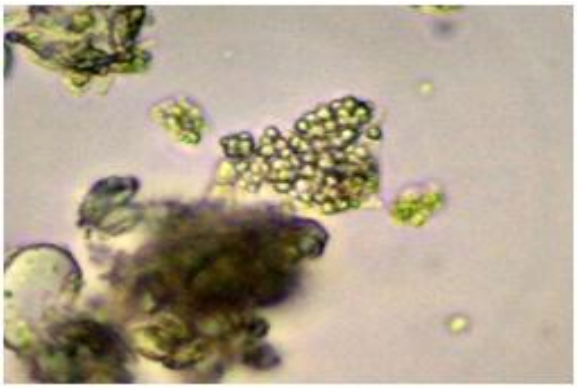

Fig 16: Starch grain of *Maricha*

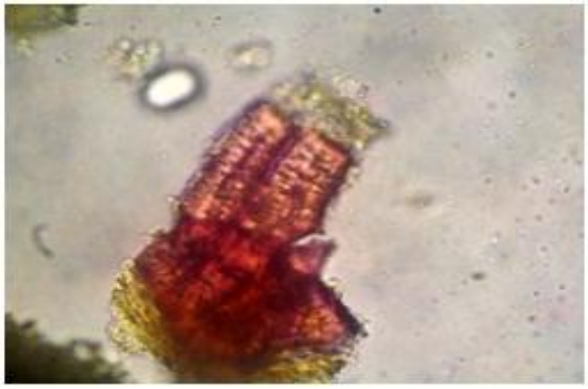

Fig 17: Beaker shaped stone cells of *Maricha*

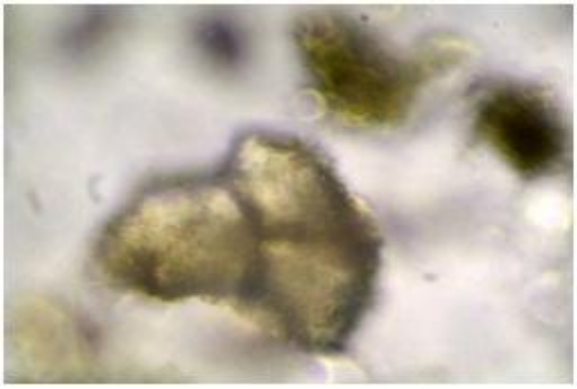

Fig 18: Black debris along with parenchyma cells of *Maricha*

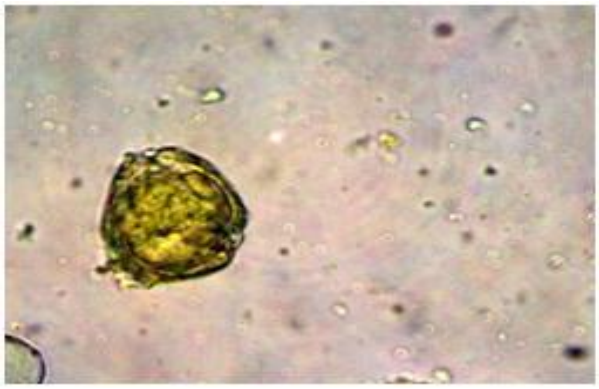

Fig 19: Pollen grain of *Nagkesara*

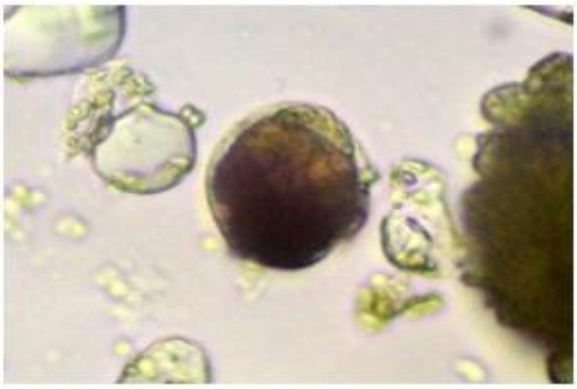

Fig 20: Pollen grain of *Nagkesara*