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# A COMPARATIVE STUDY ON PHARMACEUTICAL AND PHARMACOGNOSTICAL PARAMETERS OF VARIOUS SAMPLES IN THE PROCESS OF *CHATUSHASHTI PRAHARI PIPPALI* PREPARATION

***[1]Dr. Manjiri Jayprakash Walinjkar, [2]Dr. Mandip Goyal, [3]Dr. Harisha C. R. and [4]Dr. V. J. Shukla**

[1]Phd Scholar, Kayachikitsa, Institute of Teaching and Research in Ayurveda, Opposite B-Division Police Station, Gurudwara Road, Jamnagar- 361008.

[2]Associate Professor, Kayachikitsa, Institute of Teaching and Research in Ayurveda, Jamnagar.

[3]Head of The Department, Pharmacognosy, Institute of Teaching and Research In Ayurveda, Jamnagar.

[4]Head of The Department, Pharmaceutics, Institute of Teaching and Research in Ayurveda, Jamnagar.

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***Corresponding Author**
**Dr. Manjiri Jayprakash Walinjkar**
Phd Scholar, Kayachikitsa, Institute of Teaching and Research in Ayurveda, Opposite B-Division Police Station, Gurudwara Road, Jamnagar- 361008.

## ABSTRACT

**Background:** *Pippali* is one of the *Rasayana* drugs which are indictaed in the management of the diseases of *Pranavaha Srotasa. Chatushashti Prahari Pippali* (CPP) is made by triturating *Pippali Churna* with *Pippali Kwatha* constantly for 64 *Prahara* (192 hours) which increases the potency of *Pippali*, making it more effective. Nowadays, due to adulteration of raw drugs or the difference in the making process, it is difficult to get the authentic and good quality CPP. **Aims and objective:** The five different samples of *Pippali Churna* in the process of making CPP were analyzed in the present study through Pharmacognostical and Pharmaceutical parameters for comparing their quality and for standardization of parameters. **Material and methods:** For pharmacognostical evaluation, the five samples i.e. raw drug, two market samples of CPP, self-made mid product (100 hours of trituration), and self-made final product of CPP were studied. **Result:** All four samples of CPP showed variation in comparison with sample one i.e. raw *Pippali* at

various pharmacognostical and pharmaceutical parameters indicating that the process of trituration has its own unique potency modifying effect of the raw drug. **Conclusion:** The self-made final product of CPP is noticed as a preeminent sample for further clinical study amongst five, as stone cells, mesocarp cells were disturbed at the utmost stage in this sample releasing internal molecular contents which is an essential active component enhancing the bio-availability of the drug and thus increasing potency of the drug.

**KEYWORDS**: *Chatushashti Prahari Pippali*, Pharmaceutics, Pharmacognosy.

## INTRODUCTION

*Pippali* (*Piper longum* Linn.) is one of the *Rasayana* (~rejuvenation) drug which is indicated in the diseases of *Pranavaha Srotas* (~respiratory system).[1] *Chatushashti Prahari Pippali* (CPP) is made by triturating *Pippali Churna* (~powder) with *Pippali Phanta* (~decoction) constantly for 64 *Prahara* (~192 hours) which thus increases the potency of *Pippali* making it more effective. Textual indications of CPP are *Vata* and *Kapha* disorders, *Kasa* (~cough), *Shwasa* (~dyspnea), *Agnimandya* (~diminution of digestive fire), *Aruchi* (~tastelessness), *Amlapitta* (~hyperacidity), *Shoola* (~pain), *Kaphaja Jwara* (~fever due to dominancy of *Kapha dosha*) and *Jeerna Jwara* (~chronic fever).[2]

This study is an attempt to establish, the pharmacognostical and pharmaceutical characteristics of different samples of CPP. These results can be employed as suitable quality control measures to ensure the quality, safety, and efficacy of this drug. The parameters studied here are useful to identify and authenticate the traditionally important Ayurvedic drug CPP and this will prove helpful in the preparation of herbal monographs and pharmacopoeial standards of the concerned drug. Due to modernization, it's very difficult to get original *Pippali* as well as CPP. The pharmacognostical study is an important tool for the identification of the original crude drug and its microscopical content. Pharmaceutical study is necessary for the standardization of finished product in the market, there are so many drugs which are adulterated or having less potency due to several problems such as unavailability of good quality raw drug, adulteration in making process of finished drug to save cost or money etc. So, it's a need of an hour to get comparative analysis between various samples of raw and finished drug for comparing their quality and for standardization of parameters. In the present study, an attempt has made to assess difference between raw drug, market samples of CPP and self-made CPP regarding quality or adulteration. In Ayurvedic classics, it is quoted that if the drug is triturated with its own *Swarasa* (~juice) / *Kwatha* it becomes more potent

or effective with reduction in dosage and becomes fast acting too.[3] Thus, a comparative analysis of *Pippali* and CPP was performed to find the difference between raw drug *Pippali* and CPP. Also self-made mid-product of CPP (product obtained after 100 hours of trituration of *Pippali Churna* with *Pippali Kwatha*) and final product of CPP (product obtained after complete 64 *Prahara* i.e. 192 hours of trituration of *Pippali Churna* with *Pippali Kwatha*) was also compared to analyze variation or any further changes occurred due to the process of trituration.

So, total five different samples i.e. *Pippali*, two market samples of CPP, self-made mid-product and self made final product of CPP were taken for the comparative analysis through Pharmacognostical and pharmaceutical study.

## MATERIAL AND METHODS

### Drug Material

Sample 1 – Raw drug;

Sample 2 – First market sample, *Chatushashti Prahari Pippali*;

M.L.No.: A-1800/89, Batch: 26, Mfd.: 05/2021, Expiry.: 04/2024

Sample 3 – Second market sample, *Chatushashti Prahari Pippali*;

M.L.No.: AYU-150, Batch: P210100290, Mfd.: 01/2021, Expiry: 12/2022

Sample 4(self-made mid product) – *Pippali* after 100 hours of *Bhavana* (~pulverization by adding a liquid to a powder)

Sample 5(self-made final product)–*Chatushashti Prahari Pippali.*

### Method of Pharmacognostical evaluation

Raw drugs were identified and authenticated from ITRA, Pharmacognosy Laboratory. The identification was carried out based on the morphological features, organoleptic features, and transverse section microscopy of the individual drugs. For pharmacognostical evaluation, drugs were studied under the Carl zeiss Trinocular microscope attached with the camera, with stain, and without stain.[4] The microphotographs were also taken under the microscope.

### Organoleptic Character

The colour, taste, and odour of the samples were noted down. These characters were useful in having a primary idea about the quality of different formulations without using chemical tests. It includes sensory characters of the drug.

1. *Sparsha* (~touch)

2. *Rupa* (~appearance/ visual form)

3. *Rasa* (~taste)

4. *Gandha* (~smell)

**Physicochemical Parameters**

All physicochemical tests were done with the help of Ayurvedic pharmacopoeia of India.

**1. Particle size of various samples**

For the particle size determination, a total 100 gm were taken and passed through 60, 85,120,160 mesh size sieve. After that, the weight passed through it has measured and the percentages are calculated.

**2. Loss on drying**

The loss on drying was determined by taking 2 g, accurately weighed sample, in a dried and previously weighed petri-dish; it was spread evenly and dried in an oven at 110°C till constant weight. The weight after drying was noted and loss on drying was calculated. The percentage of loss of drying was calculated on the basis of an air-dried sample.

**3. Total ash**

The ash value of the sample was determined by incinerating about 2 g of accurately weighed drug in a tarred silica crucible at a temperature not exceeding 450°C until free from carbon. Then was cooled and weighed. If a carbon-free ash was not obtained in this way, then the charred mass was exhausted with hot water and the residue was collected on an ashless filter paper. Filter paper evaporated to dryness, and ignited at a temperature not exceeding 450°C. The percentage of ash was collected with reference to the air-dried sample.

**4. Soluble water-soluble extractive**

About 5g, accurately weighed sample was macerated with 100 ml of distilled water in a closed flask for twenty-four hours, shaking frequently for six hours, and allowed to stand for eighteen hours. It was filtered, taking precaution against loss of solvent, and 25 ml of the filtrate was evaporated to dryness in a previously weighed dried evaporating dish. First dried over a water bath and then at 110°C in a hot air oven, to constant weight and weight was noted down. The percentage of water-soluble extractive was calculated with reference to the air-dried sample.

**5. Alcohol soluble extractive**

Alcohol soluble extractive value was determined by the same procedure as described in water-soluble extractive value by taking methanol instead of water. The percentage of alcohol-soluble extractive was calculated concerning the air-dried sample.

**6. pH**

For determination of pH a 5gm sample was added to 100 ml water. The solution had shaken for 30 min and then the reading was taken with pH paper.

## OBSERVATIONS AND RESULTS

### A. Organoleptic Characteristics of *Pippali Churna*

Organoleptic characteristics of 5 samples were carried out, in which dark green color was observed in samples 1&2 while light brown color in sample 3 while blackish green color in sample 4&5, hence it be said that there was slight difference in the color of all 5 samples. In odour, pungent odour was observed in samples 1&2 while astringent in sample 3 and aromatic bitter odour in samples 4&5. In taste, pungent taste was observed in samples 1, 2, 4 & 5 while astringent was observed in sample 3. Rough touch was observed in all the samples. All these characteristics are tabulated in table no.1

### B. Particle size of various samples

Maximum particle observed in 60 mesh size was from sample A, in 85 mesh size also sample 1 had highest particle size. While sample 5 had higher particle size from 120 &>120 mesh size shown in table no.2.

### C. Ash Value

The lowest ash value is shown in sample no.3 and all other shown in table no.3.

### D. Loss on drying of various samples

The lowest LOD was found in sample no.3 and all other shown in table no.4.

### E. pH

The pH is found for all samples in acidic pH level shown in table no.5.

### F. Water soluble extractive value

The maximum water soluble extractive value was found in sample no.4 and all other shown in table no.6.

### G. Alcohol soluble extractive value

The maximum alcohol soluble extractive value was found in sample no.1 and all other shown in table no.6.

### H. Comparative Microscopic Analysis for various samples in the process *Chatushashti Prahari Pippali* preparation

The comparative microscopic analysis for all 5 samples described in Table no.8 and from Fig no. 1-5.

## TABLES

**Table No. 1: Organoleptic Characteristics of *Pippali Churna* of different samples.**

| | Colour | Odour | Taste | Touch |
|---|---|---|---|---|
| **Sample 1** | Dark Green | Strong Pungent | Pungent | Rough |
| **Sample 2** | Dark Green | Strong Pungent | Pungent | Rough |
| **Sample 3** | Light Brown | Light Astringent | Astringent | Rough |
| **Sample 4** | Blackish Green | Aromatic bitter | Pungent | Rough |
| **Sample 5** | Blackish Green | Aromatic bitter | Pungent | Rough |

**Table No. 2: Particle size of various samples in %.**

| | S1 | S2 | S3 | S4 | S5 |
|---|---|---|---|---|---|
| **60** | 54.086 | 53.336 | 43.890 | 37.530 | 22.308 |
| **85** | 33.792 | 30.396 | 33.717 | 38.121 | 28.388 |
| **120** | 9.890 | 11.028 | 15.820 | 14.121 | 35.332 |
| **>120** | 2.118 | 4.332 | 6.183 | 6.720 | 9.932 |

**Table No. 3: Ash value of various samples.**

| | S1 | S2 | S3 | S4 | S5 |
|---|---|---|---|---|---|
| % of Ash | 7.40 | 5.87 | 3.18 | 6.60 | 10.27 |

**Table No. 4: Loss on Drying of various samples.**

| | S1 | S2 | S3 | S4 | S5 |
|---|---|---|---|---|---|
| % of Loss on drying | 11.55 | 8.12 | 3.21 | 10.89 | 10.40 |

**Table No. 5: pH of various samples.**

| S1 | S2 | S3 | S4 | S5 |
|---|---|---|---|---|
| 5.980 | 6.198 | 4.58 | 5.03 | 5.08 |

**Table No. 6: Water Soluble Extract of various samples.**

| | S1 | S2 | S3 | S4 | S5 |
|---|---|---|---|---|---|
| % Extractive value | 19.45 | 18.34 | 12.44 | 20.28 | 19.16 |

**Table no. 7: Alcohol Soluble Extractof various samples.**

| | S1 | S2 | S3 | S4 | S5 |
|---|---|---|---|---|---|
| % Extractive value | 27.57 | 23.07 | 14.35 | 20.30 | 17.71 |

**Table No. 8: Comparative pharmacognostical analysis of five samples.**

| Sample 1 (Fig : 1) | Sample 2 (Fig : 2) | Sample 3 (Fig : 3) | Sample 4 (Fig : 4) | Sample 5 (Fig : 5) |
|---|---|---|---|---|
| Simple Fibre | Simple Fibre | Simple Fibrebroken | Simple Fibre (broken &distributed) | Simple Fibre (broken & distributed) |
| Starch grain present | Increased contents of simple and component Starch | Increased contents of simple and component Starch | Increased contents of simple and component Starch | Increased contents of simple and component Starch |
| Oleoresin content present inside the cell | oleoresin content came out of the cell | oleoresin contentcame out of the cell but less than sample two | oleoresin contentcame out of the cell, more than sample two | Free oleoresin contentcame out of the cell, more than sample four |
| Mesocarp cells | Mesocarp cells loosened | Mesocarp cells loosened | Mesocarp cells free and loosened | Mesocarp cells free and loosened |
| Oil globules present inside the cell | Oil globules – inside and outside the cells | Oil globules - inside and outside the cells | Oil globules - inside and more outside the cells | Oil globules – maximum outside the cells |
| Vessels Xylem | Vessels xylem | Vessels Xylem | Vessels xylem | Vessels Xylem |
| Unlignified Stone cell | Stone cell lignified and disturbed upto 20-30% | Stone cell lignified and disturbed less than sample two | Stone cell lignified and disturbed approximately upto 40-60% | Stone cells lignified and disturbed approximately upto 60-70% |
| Group of stone cells. | distributed groups of stone cells | Group of stone cells. | Group of stone cells as well as disturbed stone cells | Group of stone cells as well as disturbed stone cells |
| Black debris not observed | Black debris present | Black debris present | Disturbed Black debris | Disturbed Black debris |
| - | Lignified vessels | - | - | - |
| - | - | - | Epicarp cells | - |

## FIGURES LEGENDS

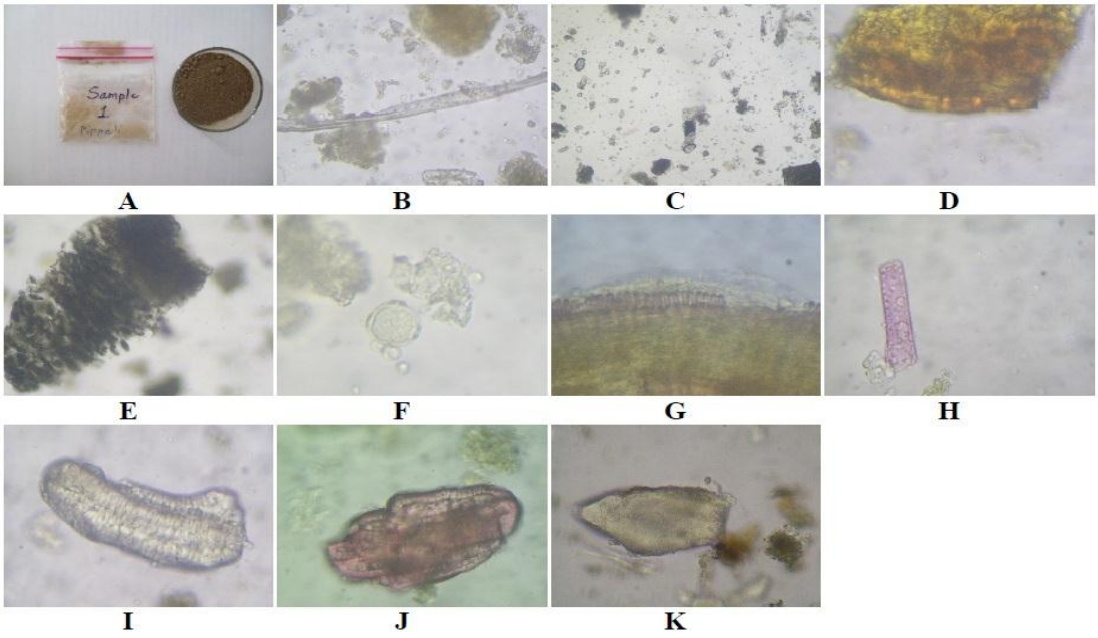


**Figure 1: Pharmacognostic profile of sample 1.**

A: Sample 1, B: Simple Fibre, C:Starch grain,D: Oleoresin content, E:Mesocarp cells, F: Oil globules, G: Vessels xylem, H: Vessels Xylem, I: Unlignified Stone cell, J: Stone cell unlignified, K: Group of stone cells.

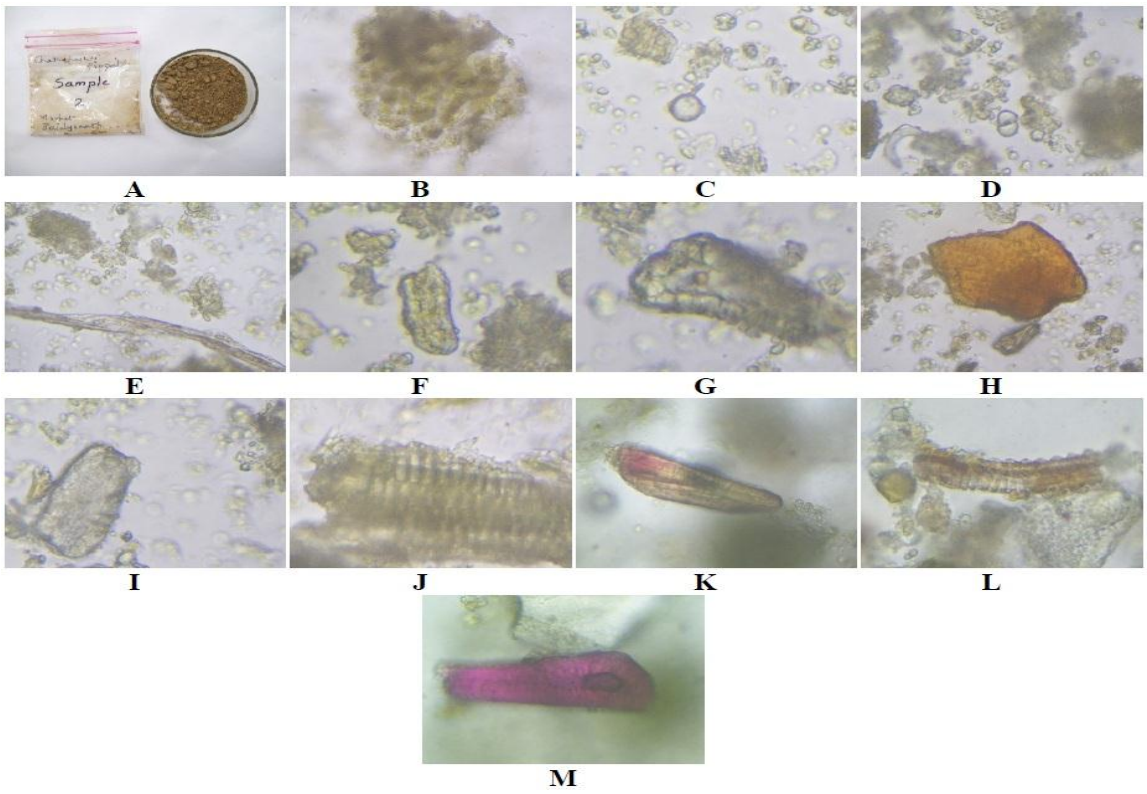


**Figure 2: Pharmacognostic profile of sample 2.**

A: Sample 2, B: Mesocarp cells, C: Starch cells, D: Stone cells distributed, E: Fibers, F: Black debris, G: Black debris, H: Oleoresin content, I: Black debris, J:Vessels, K: lignified stone cells, L:Lignified vessels, M:Lignified stone cells.

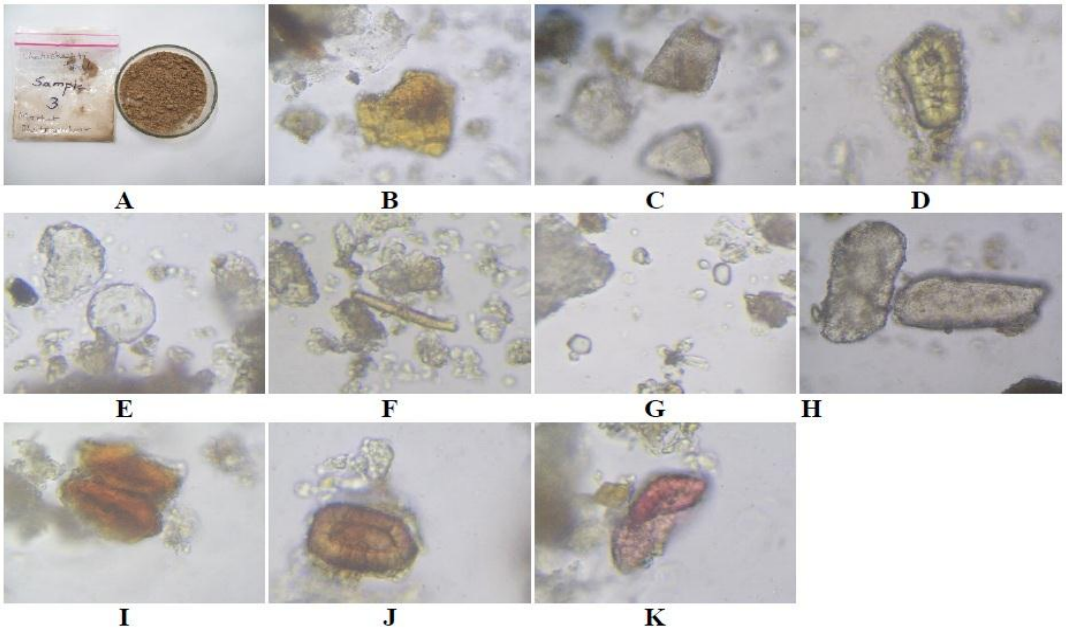


**Figure 3: Pharmacognostic profile of sample 3.**

A: Sample 3 B: Oleoresin C: Black debris D: Stone cells E:Oil globules F:Fibers G: Starch grains, H:Black debris, I:Stone cells in group, J: Lignified stone cells, K:Group of stone cells.

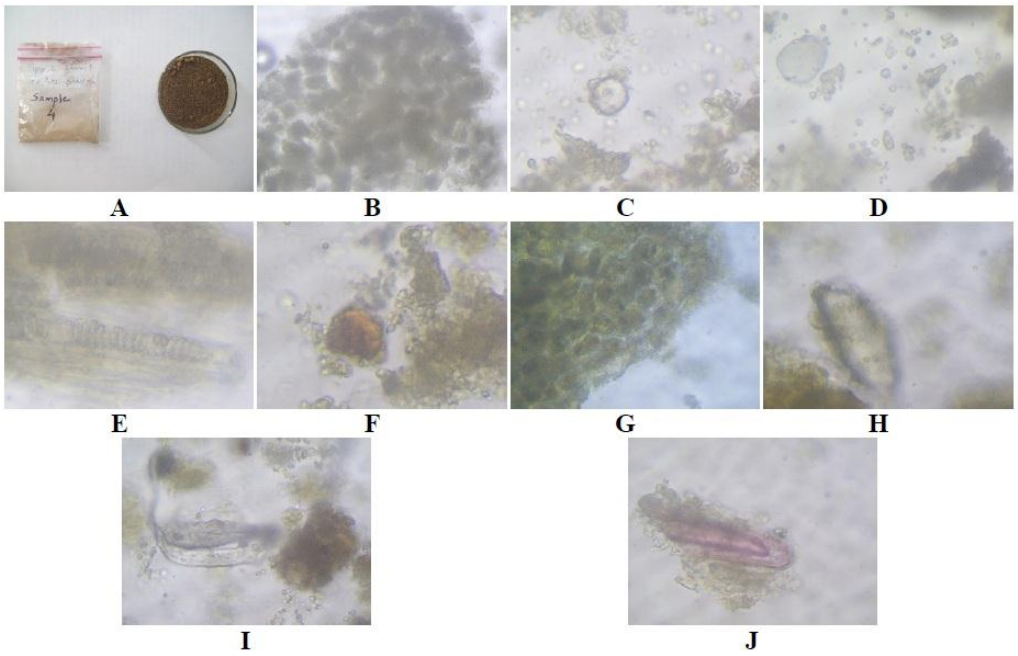


**Figure 4: Pharmacognostic profile of sample 4**

A: Sample 4, B: Mesocarp, C: Starch grains, D: Starch grains, E: Xylem vessels, F:Oleoresin, G:Epicarp, H:Disturbed Black debris, I:Fibre (broken &debris), J:Stone cells lignified.

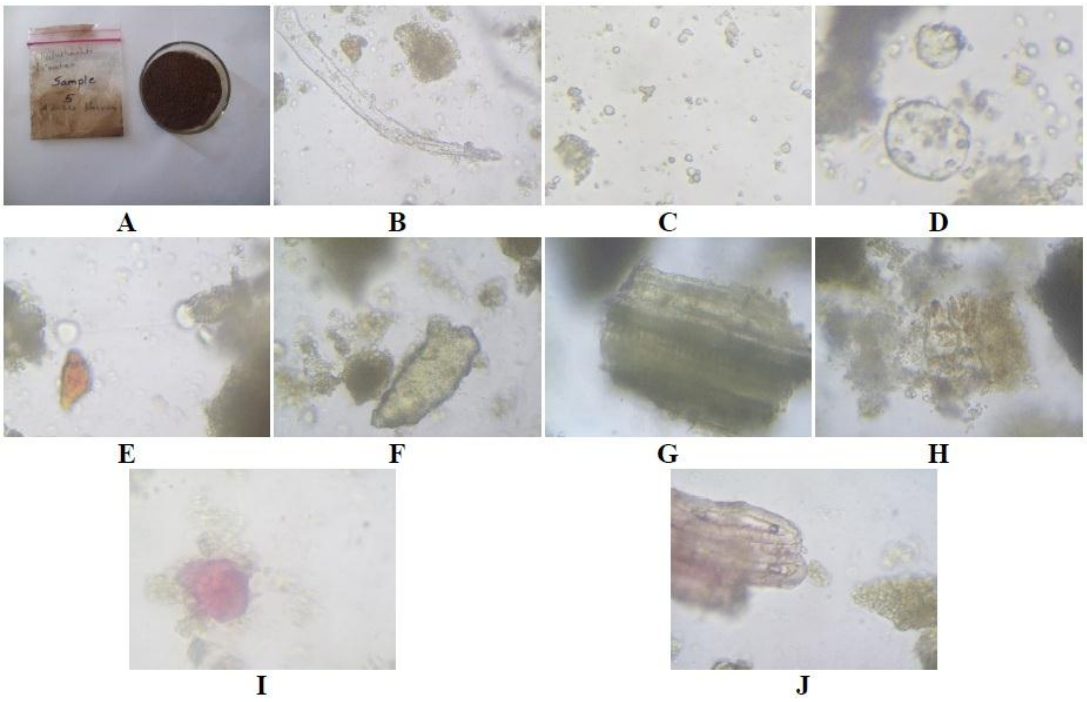


**Figure 5: Pharmacognostic profile of sample 5.**

A:Sample 5, B:Fibre,C:Starch grains, D:Oil globules, E: Oleoresins F: Black debris, G:Xylem vessels, H:Mesocarp, I:Stone cells, J: Group of stone cells.

## DISCUSSION

Microscopic characters of individual raw drugs and prepared drug show definite variation. This may be due to the effect of *Panchamahabhuta* constitution. The main interaction of *Panchamahabhuta* is lignified stone cells with simple fibres which indicate that particles may be influenced by *Vayu* and *Akash*. Function of these cells is to strengthen the central axis of plant organs mechanically against gravity, mechanical disturbances and physical damage. All five samples are having simple fibres but sample no.1 is not having lignified stone cells that mean it is not affected by *Vayu* (~air element) and *Akash* (~ether/space element). In immature fruits at times during development, it was only possible to distinguish the epicarp and mesocarp since the endocarp remains slightly differentiated. Stony endocarp became distinct when the fruit matures. In mature fruit the pericarp possessed a thin epicarp, a more or less fleshy parenchymatous mesocarp and stony endocarp.

Water moving to tracheid must pass through a thin modified primary cell wall known as the pit membrane, which serves to prevent the passage of damaging air bubbles. Vessel members are the principal water-conducting cells in angiosperms (though most species also have tracheids) and are characterized by areas that lack both primary and secondary cell

walls, known as perforations. Water flows relatively unimpeded from vessel to vessel through these perforations, though fractures and disruptions from air bubbles are also more likely. In addition to the tracheary elements, xylem tissue also features fibre cells for support and parenchyma (thin-walled, unspecialized cells) for the storage of various substances.[5,6] In the present study, Sample 1 contains Simple Fibre, Starch grain, oleoresin content, mesocarp cells, oil globules, vessels xylem, vessels xylem, unlignified stone cell, stone cell lignified, and group of stone cells. Sample 2 contains mesocarp cells, starch cells, stone cells distributed, fibres, black debris, black debris, oleoresin content, black debris, vessels, lignified stone cells, lignified vessels, Sample 3 contains oleoresin, black debris, Stone cells, oil globules, fibres, starch grains, black debris, stone cells in group, lignified stone cells, Group of stone cells. Sample 4 contains mesocarp, starch grains, starch grains, xylem vessels, oleoresin, epicarp, disturbed Black debris, Fibre (broken &debris), Stone cells lignified. Sample 5 contains fibres, starch grains, oil globules, oleoresins, black debris, xylem vessels, mesocarp, Stone cells, Group of stone cells. It is summarized in table No.8.

## CONCLUSION

In the comparative pharmacognostical and pharmaceutical analysis, sample five i.e. self-made CPP is observed better among all five samples; even also better than self made mid product which concludes that complete 192 hours of trituration make the product more efficient. The variation from market samples may indicate the adulteration of raw drug or making process. Sample five is concluded as best sample among the five samples studied, as it contains broken & distributed simple fiber, increased contents of simple and component starch, free oleoresin contents, free and loosenedmesocarp cells, oil globules – maximum outside the cells, xylem vessels, lignified and disturbed stone cells, disturbed black debris; also particle size is good in sample five as compared to other sample. All these interpretations indicate that, due to process of trituration the intra-cellular contents get free from molecular wall and came outside the cellular lining due to which the active components of the drug will be more stalwartly available on the consumption of drug increasing its bioavailability which increases its potency or efficacy from raw drug and minimize the dose and duration. Hence, the sample five can be recommended for future clinical trial for betterment of human life. The profile of sample five can also be used for standardization of CPP and in future references.

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