IJBPAS, October, 2024, 13(10): 5226-5252

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PREPARATION AND EVALUATION OF AYURVEDIC FORMULATION: PANCHKOLA GHANVATI

SINGH N., GUPTA M. AND GUPTA S.P.*

Rajiv Gandhi Institute of Pharmacy, Faculty of Pharmaceutical Science & Technology, AKS University, Satna (MP)-India-485001

***Corresponding Author: Dr. Surya Prakash Gupta: E Mail: suryatony@yahoo.co.in**

Received 24th Sept. 2023; Revised 25th Oct. 2023; Accepted 16th Jan. 2024; Available online 1st Oct. 2024

https://doi.org/10.31032/IJBPAS/2024/13.10.9107

ABSTRACT

The current research focuses on the preparation and characterisation of ayurvedic Panchkola ghan vati, while adhering to quality control protocols for both the raw components and the final product. The acquired values, which represent the ranges of physico-chemical parameters, may be used to establish new pharmacopoeial standards for ensuring consistent batch-to-batch manufacture of Panchkola ghan vati in the conventional manner. The raw material utilised for preparing Panchkola churna contains phytochemical compounds that contribute to the therapeutic effectiveness of the medicinal formulations. These ingredients also provide insights into the underlying mechanism of pharmacological activity.

Keywords: -Ayurvedic formulation, Panchkola churna, Quality control, Physico-chemical parameters

INTRODUCTION

Ayurveda, an ancient Indian system of medicine, is renowned for its substantial impact on the healthcare of human civilization. Nevertheless, there is insufficient scientific data to substantiate the justification for using these formulations in the field of healthcare **[1]**. Recently, there has been a recognition of the need of incorporating the well-established but less scientifically validated Ayurvedic system of medicine into healthcare **[2]**. The incomplete comprehension of the process and insufficient scientific evidence for certain preparation steps have contributed to the lack of standardization and quality

control in the production of Ayurvedic medicines **[3]**.

Meaning of Ayurveda: -Ayurveda is a compound noun in Sanskrit, consisting of the terms "Ayus" and "Veda." The term "Ayus" refers to the concept of life, whereas "Veda" denotes knowledge or science. Ayurveda is a phrase that signifies the understanding and study of life. Charaka, an ancient Ayurvedic scholar, said that "Ayu" encompasses the intellect, body, senses, and soul **[4]**.

Origin of Ayurveda: -Ayurveda, originating in India thousands of years ago, is widely recognised as the oldest type of healing in the world. The foundational principles of Ayurveda are derived from the Hindu scriptures known as devas, which are ancient Indian texts of profound insight. The Rig Veda, composed more than 6,000 years ago, has a collection of remedies that may assist mankind in overcoming several illnesses **[5]**.

Definition of Panchkola Churna:- Panchkola churna is a polyherbal Ayurvedic composition that is used for treating conditions such as fever and indigestion. The production of panchkola churna follows traditional procedures as outlined in the ancient source, Ayurvedic Formulary of India-1. The absence of a contemporary pharmacopoeia standard for the production of panchkola churna has resulted in inadequate processing methods. Medicines produced using conventional methods may lack the appropriate quality and uniformity from one batch to another **[6]**. Therefore, it is necessary to establish a standardization process for Ayurvedic churna that adheres to scientific parameters. This process should include the descriptive taxonomic identification of the raw medicine, assessment of its sensory characteristics, examination of its microscopic properties as a powder, analysis of its phytochemical composition, evaluation of its chromatographic pattern, and screening for microbiological contaminants **[7]**.

Ingredients

1. Pippali (*Piper longum* Linn, fruit)
2. Pippalimula (*Piper longum* Linn, stem)
3. *Chavya* (*Piper retrofractumvahl*, stem)
4. Chitrak (*Plumbago zeylanica* Linn, root)
5. Sunthi (*Zingiber officinaleRoxb*, rhizome)

The investigation was conducted within the framework of a trust, as a component of a programme aimed at evaluating and validating the traditional practices associated with the use of Ayurvedic medicines. Panchkola churna has long been used as a traditional remedy for alleviating symptoms of cold and fever. Therefore, it is necessary to standardize panchkola churna. The present study focuses on comprehensive standardization protocols for the creation of Ayurvedic medications, in accordance with the principles of good manufacturing practices (GMP). The criteria for

standardization of herbal products issued by the World Health Organisation (WHO) and the Ayurvedic Pharmacopoeia of India have been taken into account. As part of the standardization process in this study, certain in-process tests were created and conducted "**[8]**".

Usage: Panchkola ghanvati is traditional used for treatment of Anaha, Gulma, Sula, Aruchi, etc

Panchkola ghanvati: -Panchkola ghanvati is a solid dosage form (tablets) prepared from panchkola prawahi kwath formulated with the ingredient in the formulation composition given bellow.

Table 1: Formulation Compositions

S. No.	Name of ingredient	Botanical Name	Part Used	Part
1.	Pippali	*Pipper Longum*	Fruit	1 Part
2.	Pippalimula	*Pipper Longum*	Root	1 Part
3.	Sunthi	*Zingiber Offcinale*	Rhizome	1 Part
4.	Chitrak	*Plumbago zeylanica*	Root	1 Part
5.	Chavya	*Pipper Retrofractum*	Stem	1 Part
6.	Jala	Potable Water	-	16 Part

Preparation of panchkola ghan vati:-

- Take all the ingredients of pharmacopoeia quality
- Clean, wash and dried the ingredient no. 1 to 5.
- Crushed all the ingredients into pieces of 1-3 content.
- Mixed the ingredients and boiled in 16 times of potable water and reduced to 1/8 of original volume.
- Filter and concentrated further by heating the filtrate to obtained solidifying extract mass (ghan).
- Granulated and compressed to tablets of desired weight.
- Store it in air tight container to protect form light and moisture.

Raw material of Panchkola churna:-

Sunthi

Chavya

Pippali

Pippalimula

Chitrak

Panchkola churna powder:-

Pippali

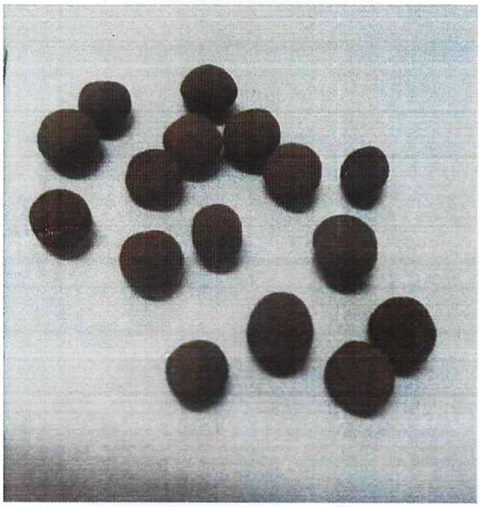
Sunthi

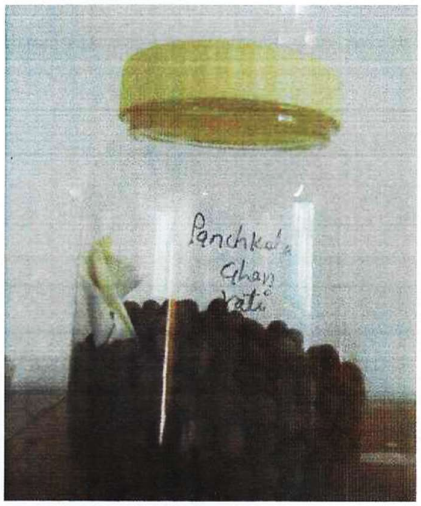
Pippalimula

Chitrak

Chavya

Panchkola ghan vati:-





Experimental Section:-

Determination of Physico-Chemical Parameters

1. LOD% (Loss on drying)

Measuring the moisture level of the medicine is crucial since it significantly contributes to preventing the development of microorganisms in the drug powder. This metric determines the quantity of volatile matter, namely the water that evaporates from the material, when the substance seems to consist only of water as the volatile component.

Procedure:-Oven method –1. Dry the dish for 30min at 105^0C.

2. Weight about 5 to 10 gm of the sample.

3.Put the sample in the oven at 105^0C for 5 hr.

4. Take the sample out of the oven and put it in the desiccators with the lid on.

5.Proceed with the process of drying, chilling, and weighing at intervals of half an hour until the discrepancy between two consecutive weighing is no more than 0.25%.

6. Record the lowest mass obtained.

2. Total Ash value

- Incinerate about 2 to 3 gm of the medicine with precision weight in a platinum and silica dish that has been weighed and crushed.
- Keep the crucible inside a muffle furnace at a temperature below 600°C until it is completely free from carbon and turns into white ash.

- Cool and weigh if it is not possible to get carbon-free ash using this method.
- Flush the burnt residue with hot water.
- Collect the remaining substance on a filter paper that does not leave any ash behind.
- Combine the filtrate and evaporate it until it becomes completely dry.
- Ignite only when the temperature is below 600°C.
- Calculate the percentage of ash relative to the medication that has been dried in the air.

3. Acid insoluble ash

- Transfer the ash collected before into a 250ml beaker without any loss, and then add 100ml of diluted hydrochloric acid (HCl).
- Wash the crucible using 10ml of acid and then transfer the washed acid to the beaker.
- Apply heat to the beaker until the liquid reaches its boiling point.
- Filter the solution and collect the insoluble materials on an ash-free filter paper.
- Rinse with hot water until the liquid passing through the filter is neither acidic nor basic.
- Move the filter paper, which contains the substance that cannot dissolve, back into the original container.
- Dry the hot plate and ignite it at a temperature of 600°C in a muffle furnace.
- Allow the residue to cool in appropriate desiccators for 30 minutes and weigh it promptly.
- Continuously repeat the technique until a consistent weight is achieved.
- Calculate the acid insoluble ash content relative to the medication that has been dried in the air.

4. Water soluble extractive

- Grind 5g of the medicine into a coarse powder and mix it with 100ml of distilled water in a sealed flask. Let it sit for 24 hours.
- Shake the mixture regularly for a duration of 6 hours.
- Let it stand for 18 hours.
- Implement a filtration system promptly to prevent water loss.
- Evaporate 25ml of the filtrate completely in a shallow dish with a flat bottom that has been weighed. Dry the dish at a temperature of 105°C until a consistent weight is achieved. Calculate the percentage of water soluble extractive in relation to the weight of the drug that has been dried in the air.

5. Alcohol soluble extractive

- In a flask that has been sealed, combine 5 gm of the drug that has been coarsely pulverised with 100 millilitres of alcohol that has a certain concentration. Allow the mixture to rest for a period of twenty-four hours.
- It is recommended to shake the mixture at regular intervals for duration of six hours.
- Allow it to remain in place for a period of 18 hours.
- To avoid the loss of solvent, it is important to filter quickly while taking measures. 25 millilitres of the filtrate are allowed to evaporate entirely in a shallow dish with a flat bottom that has been weighed before the process begins.
- Make sure that the sample is dried at a temperature of 105°C until it reaches a weight that is constant throughout.
- In order to determine the proportion of alcohol-soluble extractive to the drug that has been dried in the air, it is necessary to calculate the percentage.

Evaluation of Panchkola Ghanvati:-

1. Hardness: -The durability of tablets against chipping, abrasion, or breakage during storage, transport and handling prior to use is primarily determined by their hardness. Traditionally, a practical guideline suggested that a tablet should exhibit adequate hardness if it could break cleanly with a sharp snap when held between the second and third fingers and broken over the thumb used as a fulcrum. However, it should not break when dropped on the floor. For reasons of consistency and control, numerous efforts have been undertaken to quantify the degree of hardness of tablets more precisely. These efforts aim to standardize how tablet hardness is measured, ensuring a reliable assessment that can be uniformly applied across different batches and formulations.

2. Friability: -

This test is designed for compressed tablets and aims to assess their physical strength.

Procedure –

- For pills weighing 0.65 gm or less on average. Obtain a representative sample of intact tablets weighing about 6.5g for tablets with an average weight more than 0.65g. For tablets with an average weight greater than 0.65g, collect a sample of 10 intact tablets.
- Thoroughly remove the dust from the pills.
- Measure the precise amount of pills needed by weight.

- Put the pills into the drum and spin it 100 times.
- Take out the pills, eliminate any loose dust from them, and measure their weight with precision.
- Adjust the drum base to produce an angle of about 10 centimetres with the horizontal if the size or shape of the tablets leads to erratic tumbling.
- The pills do not adhere to each other when placed side by side, which prevents them from dropping without obstruction.

3. Disintegration test: -Procedure:-

- Fill the tank of the disintegration apparatus with distilled water up to the indicated mark.
- Measure and pour 750 ml of distilled water into each of the 1000 ml beakers.
- Set the timer on the instrument for 90 minutes and adjust the water temperature in the beaker to 37°C ± 0.5°C.
- Place one tablet or capsule into each tube of the apparatus.
- Add a disk to each tube to ensure consistent submersion of the tablets or capsules.
- Immerse the assembly in the beaker of water and start the apparatus.
- Monitor and record the time it takes for each tablet or capsule to disintegrate completely.
- The test is considered passed if all tablets or capsules disintegrate within the 15 to 30 minute timeframe.

4. pH value (10% aqueous extract):- The pH value of an aqueous liquid is defined as the common logarithm (base 10) of the reciprocal of the hydrogen ion concentration, where the concentration is expressed in grams per liter. In simpler terms, if you denote the hydrogen ion concentration as [H +] in grams per liter, the pH is calculated using the formula:

$$pH = -\log_{10}([H+])$$

This formula provides a measure of the acidity or basicity of the solution based on the activity of hydrogen ions present.

5. Uniformity of weight (+10):- This parameter refers to the uniformity of dosage and is relevant for tablets and capsules, where precise control over the weight and composition is crucial for consistent quality and efficacy. In contrast, traditional Ayurvedic and Siddha formulations like vati or gutti, which are often made by hand or with the help of simple manual machines, do not typically apply this parameter. The preparation of vati or gutti involves binding herbal or plant materials into small pills, making it challenging to achieve uniform weight across batches. The manual process and the natural

variation in the density and moisture content of the vegetable parts contribute to these inconsistencies. Consequently, the strict control of weight as a quality parameter is less feasible for these traditional formulations compared to more standardized pharmaceutical forms.

6. Microscopic character:- Microscopic identification with the help of microscope, of botanical ingredient is a standard for the identification purposes in several solid and semi solid compound formulation as well as plant raw material. Powder microscopy is done in order to identify the drug powders and to determine which plant part was used as the drug. For this purpose, a very small amount of drug was placed over the clean, dry slide and a drop or two of glycerin was added and mixed. Then it was covered with cover -slip and observed under the microscope after drying.

7. Microbial contamination:- Medicinal plant material normally carries a great number of bacteria and moulds, often of soil origin and of environment. The condition of test for microbial contamination is designed to minimize the accidental contamination of the material being examined.

a. Plate count for bacteria: - Total bacteria count of a drug is done in order to determine the number of viable bacteria present in the drug sample. Inoculum was prepared using drug powder (1g drug dissolved in 25ml sterile distilled water) and 1ml of these inoculums was mixed with 15ml of liquefied media (plate count agar - M091) in a sterilized Petri-plates. Then the plates were allowed to solidify and placed in an incubator, at 37°C for 24hr in an inverted position. After 24 hr of incubation, the colonies grown were counted.

b. Plate count for fungi: - Total fungal count was done in same way as the total bacterial count was done. 1 ml of inoculums was poured was in a sterilized Petri-plate and 15 ml of autoclaved liquefied media (potato dextrose agar M096) was poured and mixed well and allowed to solidify. The plates were then incubated at 25°C for 72hr. after colonies appeared were counted.

8. High Performance Thin Layer Chromatography (HPTLC):- HPTLC is a sophisticated & automated from of TLC technique. The method is used for separation of the components present in mixture both quantitative as well as qualitative. For qualitative analysis of medicinal plant the sufficient of quantity of ethanolic extract after dissolving in methanol was passed

through 5micro milipore membrane filter unit. About 8micro liter was applied on HPTLC plates with a TLC sampler third and was piloted by the WINCATE software 1.3.2(CAMAG, Switzerland). Methanolic dilution of a solution was prepared by dissolving 3.75mg of extract in 5.0ml. Methanol and then applied on the plates. After development of plates in appropriate mobile phase solvent system (toluene: ethyl acetate) (Chloroform: ethyl acetate: formic acid) in the ratio of (7:3) (7.5:6:0.5), the plates were scanned at particular wave length in reflectance mode with a TLC scanner third (WINCAST 1.3.2, CAMAG).

Derivatization: - In situ derivatization possibility is a strong point of HPTLC. Chemical reactions are possible in situ on the plate before or after chromatography. Both the possibility has their own advantage. However, the decision depends on sample matrix level of detection / quantification and interference present. Post chromatography derivatization is more popular technique for which several hundred references in literature are available as compared to a few for pre chromatographic derivatization. The development has been recommended. Reasons for derivatization can be:-

- Transforming non-absorbing substance in to detectable derivatives. Lowering the detection limit.
- For visualization of all sample components.
- To induced fluorescence.
- To make separation easier.
- To retain volatile compound

Procedure:

- Weight 2-4gm of sample.
- Add 50ml distilled ethyl alcohol.
- Keep it over night with occasional shaking.
- Boil for 5-10min on a water bath.
- Cool and filter.
- Concentrate the filtrate and make up to 10ml in a volumetric flask.
- Switch on the HPTLC applicator.
- Regulate the pressure of the nitrogen gas to 3.5 kg in the regulator, wash syringe with test solution.
- Fill the syringe with extract prepared above for the quantitative analysis.
- Program the applicator as directed.

9. Method for preparation of microscopic slide:-To prepare and analyze vati samples using various mounting techniques, follow this detailed process:

1. **Sample Preparation:**
    - Weigh approximately 5 grams of vati.

- Triturate the vati with water in a mortar to form a paste.

2. **Initial Separation:**
    - Transfer the triturated mixture to a beaker.
    - Stir the mixture with a glass rod to ensure thorough mixing.
    - Gently swirl the beaker to allow solids to settle.
    - Decant the supernatant (the clear liquid above the settled matter) into another beaker, ensuring that the solids remain in the original beaker.
3. **Mounting and Staining Procedures:**
    - **For Chloral Hydrate Preparation:**
        - Take a small quantity of the residual solid and mix it with a few milligrams of chloral hydrate solution.
        - Rinse the mixture in water to remove excess chloral hydrate.
        - Mount the cleaned sample in glycerin for microscopic examination.
    - **For Iodine and Potassium Iodide Solution Preparation:**
        - Treat a few milligrams of another portion of the solid with iodine in potassium iodide solution. This will stain components like starches, making them easier to identify.
        - Rinse the stained sample in water to remove excess iodine solution.
        - Mount the sample in a 2% aqueous solution of potassium hydroxide to enhance visibility of certain cellular components.
        - Finally, wash the sample again in water and mount it in glycerin.
4. **Microscopic Examination:**
    - Observe the mounted samples under a microscope.
    - Note the characteristic features visible in each type of mount, such as cell structure, presence of starch

granules (which stain blue-black with iodine), and other identifiable particulates.

1. **Sunthi (*Zingiber officinale*):-** Those parenchymates cells that contain oleo-resin, starch grains that range from oval to round and are no less than 15 micron to 30 micron, and many that are up to 70 micron in size, with a hilum that is eccentric, lamellae that are distinct pitted septate fibres, and indentation on their walls.
2. **Pippali fruit (*piper longum*):-** lignified spindle shaped stone cell with wide lumen associate with vascular elements, round to oval starch measuring 3 to 8 micron diameter, some are having 3 to 4 component, fragment of thin walls cell having redish brown content starch grain.
3. **Pippalimula (piper longum):-** Short covering trichrome, long trachoid various shape and size with pits, collenchymas round to oval thick walled, field with starch grain simple and compound having 2 to 7 component round to oval Parenchymates cell crossed Septate fiber and containing starch grain.
4. **Chitrak (*Plumbago zeylanica*):-** Fragment of tangentially cut modularly ray, fragment of parenchyma showing brown content and starch grain pitted in wall lignified long fiber isolated or in grouped.
5. ***Chavya* (*piper retrofractum*):-** Various shape and size of stone cells with pitted walls wide lumen and containing with pits, round oval to rectangular thin walled, Parenchymates cell with prominent intercellular spaces.

Phytochemical test:-

Alkaloids

- Definition: Alkaloids are basic nitrogen atoms-containing natural compounds. They are synthesized by bacteria, fungi, plants and mammals and act as pharmacological agents. Examples include caffeine (found in coffee), barbiturates, and cocaine.
- Wagner's Test: To test for alkaloids, add a few drops of Wagner's reagent to 1 ml of an acidified (1% HCL v/v) alcoholic extract. The formation of a yellow or brown precipitate indicates the presence of alkaloids.

Carbohydrates

- Fehling's Test: Mix 2 ml of an aqueous extract with 1 ml of Fehling's solution 'A' and 'B', then boil for a few minutes. A brick-red precipitate indicates the presence of carbohydrates.

Flavonoids

- Test for Flavonoids: Add 5-10 drops of HCl to 0.5 ml of an alcoholic extract, followed by a piece of magnesium metal. The development of a pink, reddish-pink, or brown color indicates the presence of flavonoids.

Resins

- Test for Resins: Dissolve 1 ml of an aqueous extract in 2 ml of acetone, then pour this solution into a tube containing 2-3 ml of distilled water. The appearance of turbidity indicates the presence of resins.

Steroids

- Characteristic: Steroids are a group of structurally related compounds, mostly saturated and colorless, found in both plants and animals. They contain a perhydro-1, 2-cyclopentaphenanthrene ring system and include sterols, bile acids, sex hormones, and adrenocorticoid hormones.

Proteins

- Biuret Test: Add 5-8 drops of 10% NaOH solution and 1-2 drops of 3% CuSO4 solution to 1 ml of a hot aqueous extract. The formation of a violet color indicates the presence of proteins.

Saponins

- Test for Saponins: Add a drop of NaHCO3 to 5 ml of an aqueous extract. The test is positive if foaming occurs, indicating the presence of saponins.

Starch

- Test for Starch: Dissolve 0.015g of iodine and 0.075g of KI in 5 ml of distilled water. Add 2-3 ml of an aqueous extract to this solution. The production of a dark blue color indicates the presence of starch.

Tannins

- Test for Tannins: Add a few drops of 5% FeCl3 to 1-2 ml of an aqueous extract. A green color indicates the presence of all tannins, while a brown color suggests the presence of specific types of tannins.

RESULTS:

A. Study of Organoleptic Characters:-

1. Colour –Light brown
2. Odor – Astringent
3. Taste – better

B. Determination of Physico- chemical Parameters

1. Apparatus name – Hot Air Oven

Table 2: LOD (Loss on drying):

S. No.	Wt. of empty petridish	Wt. of petridish + 1g sample	After 5hr wt.(petridish)	After 1/2hr post wt. (petridish)	Difference
1.	16.4671	17.4671	17.4037	17.4037	0.0634
2.	16.7620	17.7620	17.701	17.7035	0.0585
3.	16.0332	17.0332	16.9649	16.9664	0.0668

Sample weight = 1gm

Average weight difference = (0.0634+0.0585+0.0668)/3

= 0.1887/3

= 0.0629

LOD % = (average weight difference x 100)/sample weight

= (0.0629x100)/1

= 6.29%

Thus the LOD value of given panchkola ghan vati sample was found to be 6.29%

2. Total ash value of panchkola ghan vati:

Apparatus name – Muffle furnace

Sample weight -2gm

Table 3: Total ash value of Panchkolaghan vati

S. No.	Crucible weight (gm)	Lead weight(gm)	First wt. (gm)	Second wt.(gm)	Third wt.(gm)	Difference
1.	17.5230	19.0734	17.6320	17.6315	17.6315	0.1085
2.	20.4910	18.9505	20.5991	20.5971	20.5970	0.106
3	18.0886	21.0203	18.1972	18.1970	18.1970	0.1084
4.	16.5370	185.3737	16.6527	16.6525	16.6522	0.1152
5.	18.1686	19.7974	18.2839	18.2838	18.2835	0.1149
6	18.5099	18.8316	18.6246	18.6242	18.6242	0.1143

Average weight difference = (0.1085+0.106+0.1084+0.1152+0.1149+0.1143)/6

= (0.6673)/6

= 0.1112gm

Total ash value = (average weight difference X 100)/ sample weight

=(2.1112X100)/2

=5.56%

Thus the total ash content of given panchkola ghan vati sample was found to be 5.56%

2. Acid insoluble ash:

Table 4: Acid insoluble ash

S. No.	Crucible weight (gm)	Acid insoluble weight (gm)	Difference
1.	17.5230	17.5414	0.0184`
2.	20.4910	20.5105	0.0195
3.	18.0886	18.1095	0.0209

Average weight difference = (0.0184+0.0195+0.0209)/3= (0.0588)/3

= 0.0196gm

Acid insoluble ash value = (average weight difference x 100)/sample weight

= (0.0196x100)/2

= 0.98%

Thus the acid insoluble ash content of given panchkola ghan vati sample was found to be 0.985

3. Water soluble extract:

Table 5: Water Soluble extract

S. No.	Weight of empty petridish (P)	Volume of sample extract	Weight after evaporation (P1)	Weight difference(P1-P)
1.	37.2110	10ml	37.3420	0.131
2.	37.4107	10ml	37.5106	0.099
3.	38.4175	10ml	38.5285	0.111

Average weight difference = (0131+0.099+0.111)/3=0.113gm

Water Soluble extract % = average weight difference x 500 = 0.113x500

= 56.5%

Thus the water soluble extract value of given panchkola ghan vati sample was found to be 56.5%4

4. **Alcohol soluble extract:**

Table 6: Alcohol soluble extract

S. No.	Weight of empty petridish (P)	Volume of sample extract	Weight after evaporation (P1)	Weight difference (P1)-P
1.	34.1920	10ml	34.2166	0.0246
2.	36.4792	10ml	36.5041	0.0249
3.	30.6867	10ml	30.7115	0.0248

Average weight difference = (0.0246+0.0249+0.0248)/3 =0.024gm

Alcohol soluble extract % = average weight difference x 500 = 0.0247x500 = 12.35%

Thus the ASE value of given panchkola ghanvati sample was found to be 12.35%

C. Photochemical screening of panchkola ghanvati:

Table 7: Phytochemical screening of panchkola ghanvati

S. No.	Name of constituent	Observation	Result
1.	Alkaloids	-	
a.	Wagner' s test	Light brown appearance	Present
2.	Tannins	Green color appearance	Present
3.	Carbohydrate:	-	-
a.	Benedict test	Violet color appearance	Present
b.	Fehling test	Red brick color appearance	Present
4.	Flavonoids	white color appearance	Absent
5.	Resins	Turbidity appearance	Present
6.	Steroids	Red color produced	Present
7.	Proteins	-	-
a.	Biuret test	Grey color appearance	Absent
8.	Saponins	Honey comb like forth	appearance
9.	Starch	Dark blue color appearance	appearance

D. Evaluation of Ghanvati

1. Hardness: - The Hardness test of panchkola ghan vati was found to be = 8kg/sec^2

2. Friability: - Before friability weight of panchkola ghan vati (20vati)= 6.1892gm

After Friability weight of panchkolaghan vati (20vati) = 6.1784gm

3. **Disintegration test:-** The Disintegration time of panchkola ghan vati was found to be =20min, Temperature set = 37^0C+_2^0C

4. pH(10%aqueous extract) The pH Value of 10% aqueous solution of ghanvati was found to be =5.65

Table 8: Determination of microbial load in panchkola ghan vati:-

S. No.	Microorganism	Observations	Permissible limits API
1.	Total bacterial count	168cfu/g	10^5/g
2.	*E. coli*	Absent	Absent
3.	*Salmonella*	Absent	Absent
4.	*Pseudomonas*	Absent	Absent
5.	*Staphylococcus*	Absent	Absent
6.	*Yeast and moulds*	135cfu/gm	10^3/g

Fig.1 E.coli (sample)

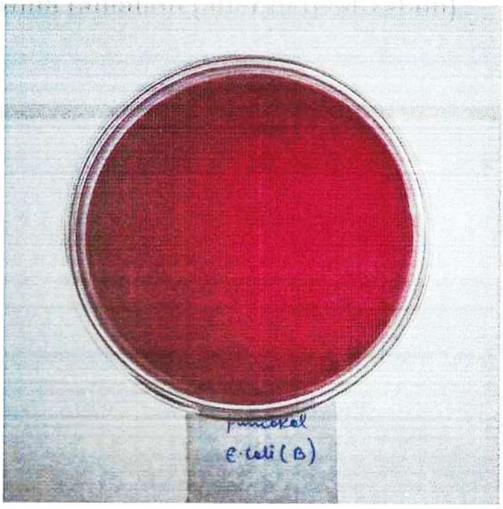


Fig.2 E.coli (blank)

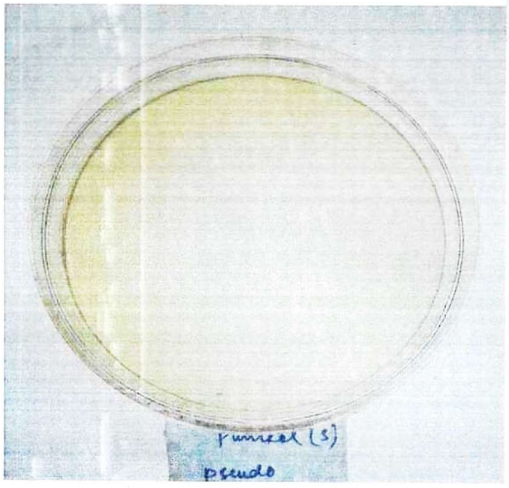


Fig.3 Pseudomonas (sample)

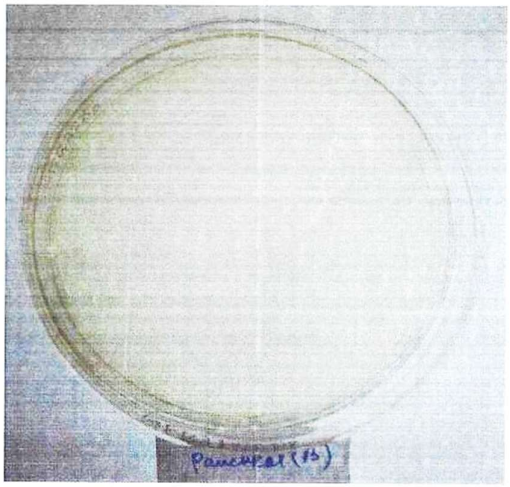


Fig.4 Pseudomonas (blank)

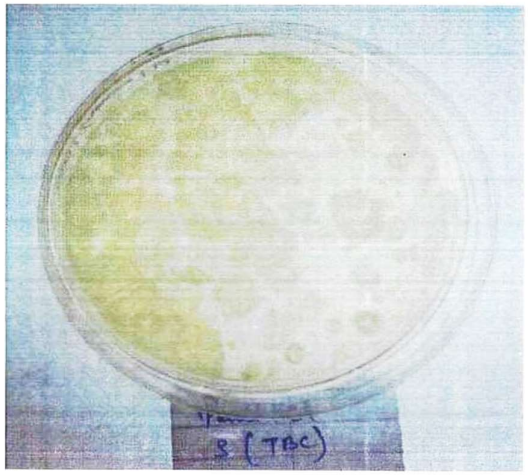


Fig.5 TBC (sample)

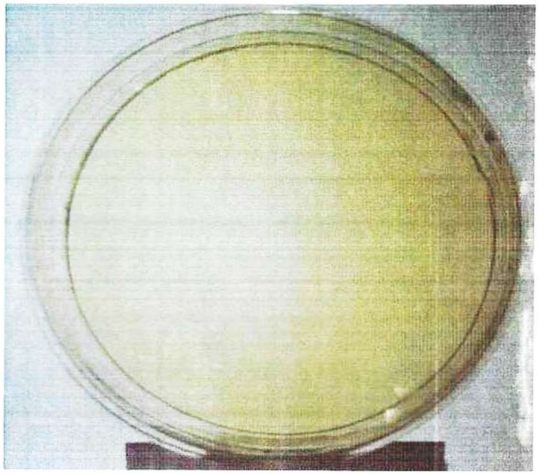


Fig.6 TBC (blank)

Fig.7 Yeast & mould (sample)

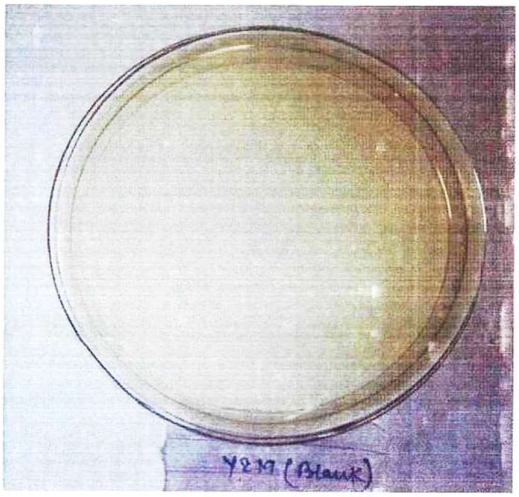


Fig.8 Yeast & mould (blank)

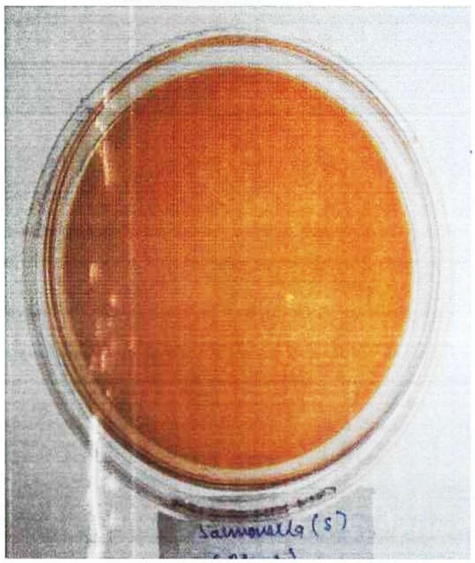


Fig.9 Salmonella (sample)

Fig.10 Salmonella (blank)

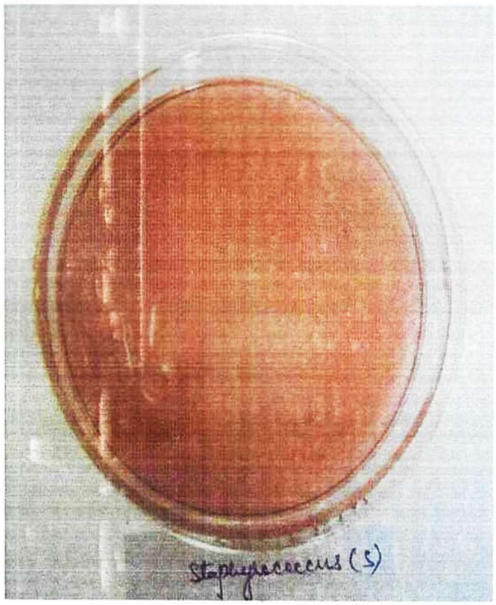


Fig.11 Staphylococcus (sample)

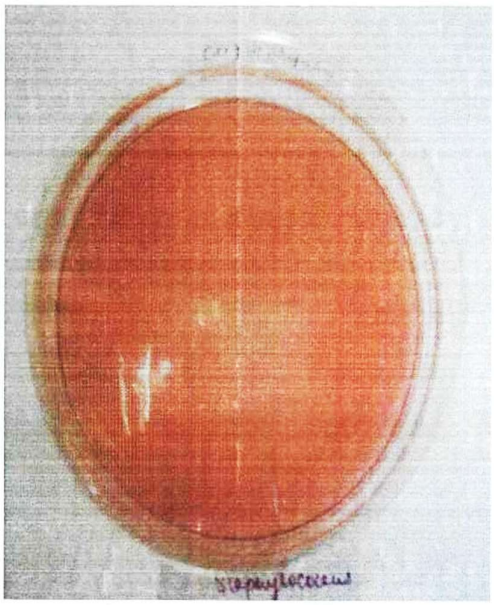


Fig.12 Staphylococcus (blank)

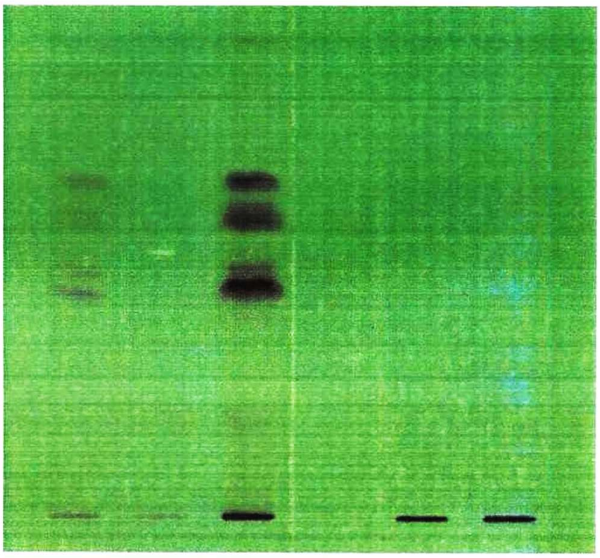
F. HPTLC Profile of panchkola ghan vati:

Solvent system – Toluene : ethyl acetate

Ratio of solvent system - (7:3)

Spray Reagent - 5% Methanolic sulphuric acid reagent

a. 254nm (Before derivatization)



A B C D E F

Sample A: vati (panchkola ghan)

Sample B: Sunthi

Sample C: pippali (fruit)

Sample D: Pippalimula

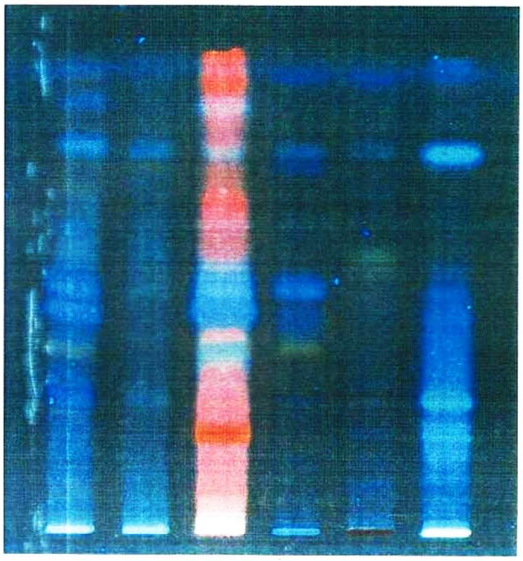
Sample E: **Chitrak**

Sample F: **Chavya**

Table No.9 Rf- values (254nm):

Sample name	Rf-1/C	Rf-2/C	Rf-3/C	Rf-4/C	Rf-5/C
Vati	0.31/black	0.45/l.black	0.49/l.black	0.59/f.black	0.66/f.black
Sunthi	-	-	-	-	-
Pippali	0.28/l.black	0.45/l.black	0.49/d.black	0.59/black	0.66/black
Pippalimula	0.49/l.black	0.59/l.black	-	-	-
Chitrak	-	-	-	-	-
Chavya	0.12/l.black	0.28/l.black	0.45/f.black	0.66/f.black	-

b. 366nm (before derivatization)



Sample A: vati (panchkola ghan)

Sample B: Sunthi

Sample C: pippali (fruit)

Sample D: Pippalimula

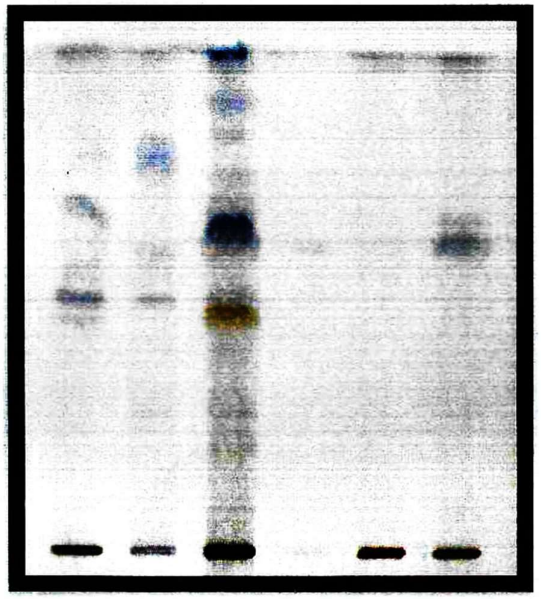
Sample E: **Chitrak**

Sample F: **Chavya**

Table No.10 Rf - values (366nm)

Sample name	Rf-1/C	Rf-2/C	Rf-3/C	Rf-4/C	Rf-5/C	Rf-6/C	Rf-7/C
Vati	0.18/l.b.	0.22/bl	0.29/flo	0.38/y.b	0.49/f.b.	0.56/y.b.	0.78/f.b.
Sunthi	0.29/l.b.	0.49/f.b	0.78/f.b	-	-	-	-
Pippali	0.22/red	0.29/l.p.	0.38/y.b	0.49/f.b	0.66/red	0.78/f.b.	0.86/p.b.
Pippalimula	0.29/blu	0.38/y.b	0.45/blu	0.49/f.b	0.78/l.b.	0.92/f.b.	-
Chitrak	0.11/w.b	0.56/y.b	0.78/l.b	0.92/f.b	-	-	-
Chavya	0.06/f.b.	0.13/l.b	0.22/l.b.	0.28/flu	0.78/flu	0.92/l.b.	-

D.White radiation (after derivatization)



Sample A: vati (panchkola ghan)

Sample B: Sunthi

Sample C: pippali (fruit)

Sample D: Pippalimula

Sample E: Chitrak

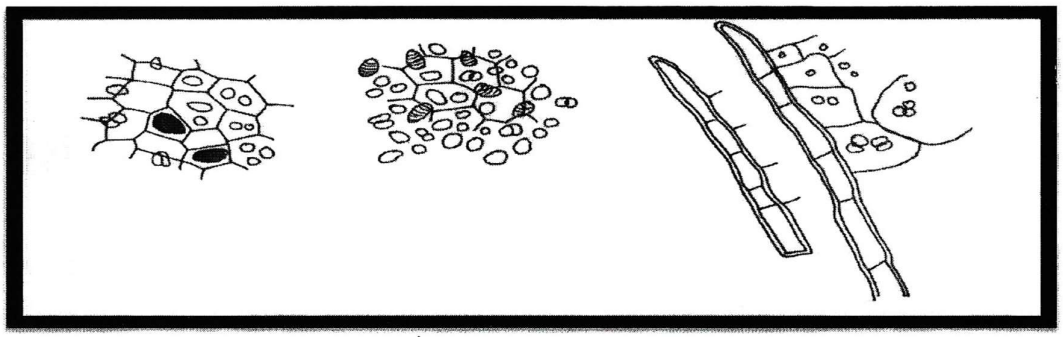
Sample F: Chavya

TABLE No.11 Rf – value (after derivatization)

Sample name	Rf-1/C	Rf-2/C	Rf-3/C	Rf-4/C	Rf-5/C	Rf-6/C	Rf-7/C
Vati	0.11/l.b.	0.44/y.g.	0.43/blu	0.63/blu	-	-	-
Sunthi	0.11/l.b.	0.28/l.b	0.47/l.b.	0.56/l.b.	0.72/blu	-	-
Pippali	0.10/l.bk	0.21/y.g.	0.28/l.b.	0.46/y.g.	0.61/blu	0.76/l.y.	0.83/l.bl
Pippalimula	0.49/l.bk	0.56/l.bl	-	-	-	-	-
Chitrak	-	-	-	-	-	-	-
Chavya	0.57/l.bl.	0.62/l.bl	-	-	-	-	-

G. Microscopy of panchkola ghan vati profile:-

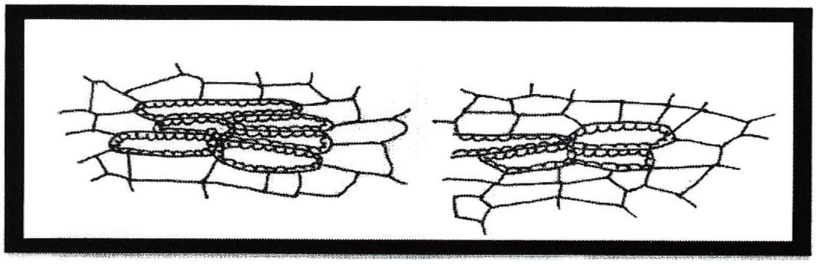
1. Sunthi (Zingiber officinale)



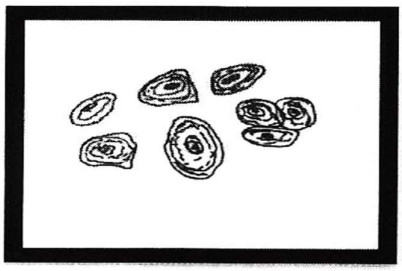
A. B. C.

A. Parenchyma containing oleo – resin
B. Starch grain
C. Septate fibers

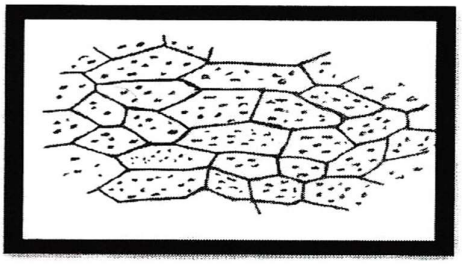
2. Pippali (piper longum)



A.



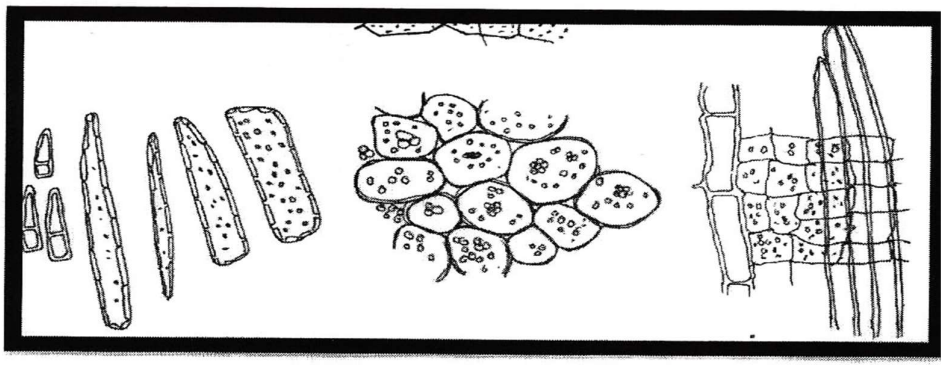
B.



C.

A. lignified spindle shaped stone cells
B. starch grains
C. fragment of thin walled cells

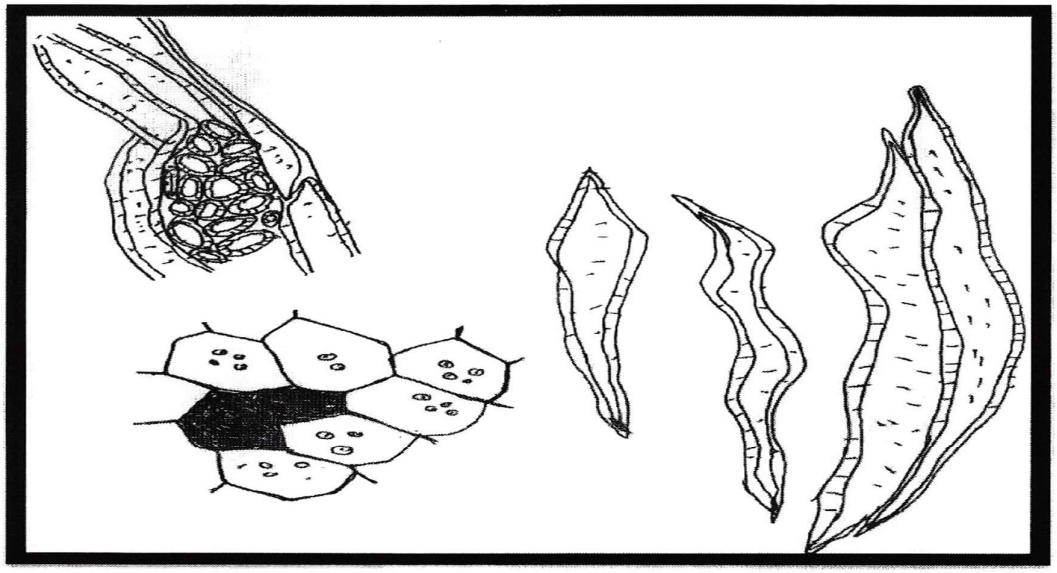
3. Pippalimula (piper longum)



A. B. C.

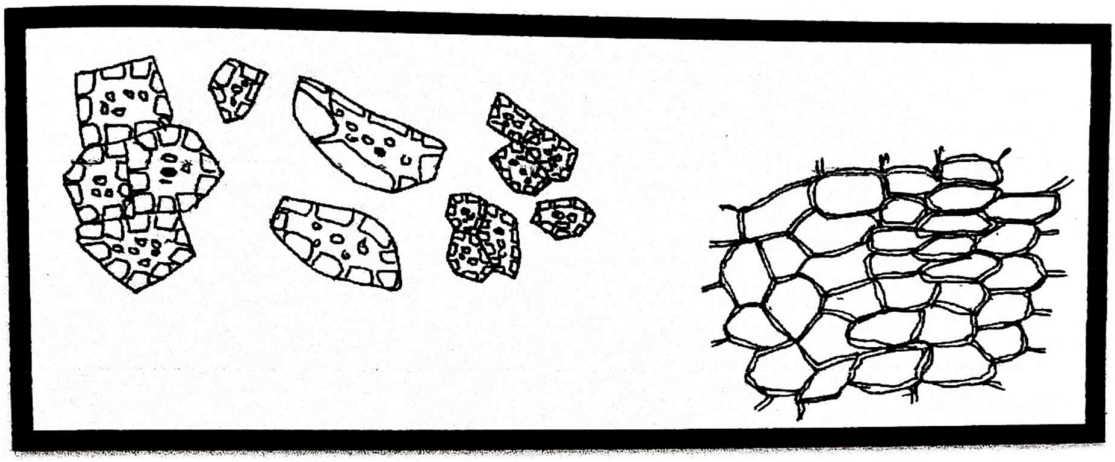
A. Covering trichoma
B. Parenchymates cells
C. Fiber with parenchyma

4. Chitrak (Plumbago zeylanica)



A. B. C.

A. Tangentially and modularly rays
B. Parenchymates cells containing brown content
C. Long Septate fiber



A. Group of stone cells.
B. Round to oval parenchymates cells.

CONCLUSION

The Panchkola compound formulation powder appears dust-like, characterized by a distinctively spicy odor and flavor. This powder can completely pass through a 355-micron mesh, with at least fifty percent able to pass through a 180-micron mesh. Its microscopic characteristics include stone cells and perisperm cells containing starch grains, fragments of parenchyma, and oil globules from Pippali; parenchymatous cells, reticulate thickening, simple pitted vessels, and fibers from *Chavya*; cork cells, fiber fragments, parenchymatous cells filled with starch grains, pitted xylem fibers with reticulate and spiral thickening, and oleo-resin canals from Sunthi; scalariform thickening, starch grains, pitted parenchymatous cells, and xylem fibers from Pippalimula; and polygonal suberized cork, reticulate and simple pitted vessels, polygonal parenchymatous cells filled with starch grains, and elongated parenchymatous cells with starch grains from Chitrak. Analytical parameters of the physicochemical data include average total ash content at 5.56%, acid insoluble ash at 0.985%, alcohol-soluble extractive at 12.35%, water-soluble extractive at 56.5%, loss on drying at 105°C at 6.29%, and a pH value of 5.05.

Conflict of Interest

The authors have no conflicts of interest.

ACKNOWLEDGMENT

The authors would like to thank AKS University, Satna, M.P. for motivation in publication of this work in a reputed Journal.

REFERENCES

[1] Chamundeeswari D, Kanimozhi P, Kumar V, Maheswara UC, Reddy A, formulation and evaluation of churna for digestive property, *Sri*

Ramchandra Journal of Medicine, 10(6) (2007) 39-43.

[2] Bahuguna Y, Saqib J, Praveen Kumar, Badola A, Evaluation and Standardization of Marketed Polyherbal Formulation-ArogyaVati, *International Journal of Ayurveda Research*, 3(2) (2013),55-64.

[3] Shukla K, Dwivedi M, Kumar N, Pharmaceutical preparation of SaubhagyaSunthi Churna An herbal remedy for puerperal women,*International Journal Ayurveda Research*. 1(1) (2010) 25-29.

[4] Rajput S, tripathi M, Tiwari AK, Dwivedi N, Tripathi SK, Scientific evaluation of panchkolachurna - an ayurvedic poly-herbal drug formulation, *Indian Journal of Traditional Knowledge*, 11(4) (2012) 697-703.

[5] Tambekar DH, Dahikar SB, exploring antibacterial potential of some ayurvedic preparation to control bacterial enteric infection, *Journal of Chemical and Pharmaceutical Research,*2(5) (2010) 608.

[6] Joshi D, Shekhar TC, Badola A, Formulation and Evaluation of AyurvedicGutika/Vati, *Indian Journal of Pharmaceutical Edition*, 2(4) (2013) 54-60.

[7] Patwardhan B, Warude D, Pushpangadan P, Bhatt N, Ayurveda and traditional Chinese medicines A comparative overview, *Journal of Chemical and Pharmaceutical Research,* 2(4) (2005) 465-473.

[8] Dorman HJ, Deans SG, Antimicrobial agent from plants: antibacterial activity of plant volatile oils, *Indian Journal of Pharmaceutical Research*88(2) (2000) 308-316.

[9] Samantha MK, Mukhargee PK, Development of natural product, *Journal of Chemical and Pharmaceutical Research,*43(7) (2000) 23-24.

[10] Mukhargee PK, Clinical Research and Regulatory Affairs, *International Research Journal of Pharmacy*, 20(9) (2003) 249-264.

[11] Patra K, Singh B, Comparative Standardization of a Poly -herbal Formulation, *Indian Journal of Traditional Knowledge,*10(4) (2011) 608-611.

[12] Anonymous the Ayurvedic Formulation of India, part 1 (Government of India, ministry of health and family welfare, New Delhi), (1996) 55-59.

[13] Anonymous the Ayurvedic Formulation of India, part-2 (Government of India, ministry of health and family welfare, New Delhi), (2000) 123.

[14] Anonymous, Indian Pharmacopoeia, (government of India, New Delhi), Quality Control Manual for Ayurvedic, Siddha and Unani Medicine, (1996).

[15] India, department of AYUSH, Ministry of Health and Family Welfare, PLIM, Ghaziabad, (U.P.) (2008) 1-99.

[16] Rostogi S, Khatoon S, Rai., Agnihotri AK, Rawat AKS, Mehrotra S, Evaluation of Ayurvedic Compound Formulation Naraca churna, Indian Journal Traditional Knowledges, 42(2) (2005) 185.

[17] World Health Organization (WHO), Quality Control Method for Medicine Plant Material, (1998) 63.

[18] Lavekar GS, Padhi MM, Pant P, Sharma MM, Verma SC, Laboratory Guide for the Analysis of Ayurveda and Siddha Formulation, Department of AYUSH, Ministry of Health and Family Welfare Govt. of India, New Delhi, (17)(2010) 23-120.

[19] Protocol for Testing of Ayurvedic, Siddha, Unani, Medicine, Published by Department of AYUSH, Ministry of Health and Family Welfare Govt. of India, (2007).

[20] Protocol for Testing of Ayurvedic, Siddha, Unani, Medicine, Published by PLIM, Ghaziabad Govt. of India Department of Ayush Ministry of Health and Family Welfare, (2007).

[21] Arthur VI, A Textbook of Quantitative in Organic Analysis Including Elementary Instrumental Analysis, Third Edition.

[22] Remington, the Science and Practice of Pharmacy, Volume-first 20th edition.

[23] Bahl BS, Tuli GD, Bahl A, Essential of Physical Chemistry (A textbook for B.Sc. student of Indian university), twenty fourth edition (revised) (2001).

[24] Aggrawal SS, Paridhavi M, Herbal Drug Technology, (2007).

[25] Ayurvedic Pharmacopoeia of India, Part First, Volume Sixth, Ministry of Health Government of India, (2008).

[26] Bajpai KS, Mishra RN, Systematic Practical Chemistry, volume first, Anusandhan Prakasan, (1999).