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Research Article

STABILITY STUDY OF *PASHANBHEDADI CHURNA* USED IN TREATMENT OF *ASHMARI* (RENAL CALCULI): WITH RESPECT TO BASELINE MICROBIAL DIAGNOSTIC MODALITIES

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ABSTRACT

Ashmari (Renal Calculi) is most common disease of urinary system. *Ashmari* or calculus looks like small gravels/stones hence they are termed as *Ashmari.* For the treatment of *Ashmari Pashanbhedadi Churna* was taken as a trial drug. As *Pashanbhedadi Churna* was trial drug it was necessary to check the stability. Stability of the drug is the time period from the drug production until the time it is intended to be consumed. So, present study was carried out to know the stability of *Pashanbhedadi Churna* and to check microbial contamination in the *Pashanbhedadi Churna* at different time interval. *Pashanbhedadi Churna* was stored in plastic bag. Microbial study of the drug was done at different climatic conditions, humidity and temperature set ups with regular intervals for a period of 11months to analyse mycological and bacteriological findings by wet mount preparation and Gram stain test respectively. Though in different climate, temperature and humidity conditions, at the end of microbial study, *Churna* has shown absence of microbes for approx.11 months of preparation of drug. So, it is showed that drug is stable in minimum 32°C temperature to maximum 38°C and minimum humidity 23% to maximum 74% humidity. That means stability duration of drug after preparation is approx. 11 months which showed that drug was in a standard condition. Hence it is concluded that stability test of *Pashanbhedadi Churna* with respect to microbiological findings was negative at room temperature, warm and cold, dry and humid conditions.

INTRODUCTION

Ashmari (Renal Calculi) is one among eight *Mahagada* (eight dreadful disorder) mentioned by Acharya Sushruta.[1] It is the third most common affliction of the urinary tract. Description of *Ashmari* is found in almost all *Samhitas* of Ayurveda for example either as a type of *Mootraghata* (Acharya Charaka) or as a separate disease (Acharya Sushruta). An exuberance or preponderance of the deranged *Kapha* should be understood as the underlying cause of all invasions of this disease. It manifest in those individual who do not undergo purification (*Samsodhana*) of the internal channels of his organs regularly or is in the habit of taking undesirable and unsuitable (*Apathyakari*) foods and activities causing aggravation of *Kapha*.[2] This *Kapha* enters into the urinary bladder and get combines with the urine and becomes saturated with the stone forming substances and gives rise to the formation of concretion or gravels.

Acharya Sushruta and other Acharyas have given many conservative formulation for *Ashmari*, while with conservative treatment Acharya Sushruta has also given surgical treatment for *Ashmari*. Medicinal treatment includes use of various *Ghrita, Kwatha* (decoction), *Churna* (powder), *Kshara dravyas*. In modern science also urolithiasis is treated with analgesic, diuretics, allopurinol, citrate etc. In larger stones surgical treatment like PCNL, ESWL, ureteroscopy are required. In present study, *Pashanbhedadi Churna* was used to treat *Ashmari* (Renal Calculi) which is mentioned by Acharya Charaka. For the first time the research work was carried out for its authentication and microbial study.

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This drug was prepared in pharmacy of Gujarat Ayurved University, Jamnagar by adopting standard operative procedure for *Churna* formation.

There was no any preservatives added to the test drug. Drug preparation was finished on 19/02/2021. Finished product was kept in airtight plastic bag at room temprature.

It was essential to prepare the formulation in a better form to avoid microbial contamination. Stability of a pharmaceutical product is the capability of a perticular drug in a specific container, to remain within its physical, chemical, microbiological and therapeutic efficacy. Thus in the present study, attempt was taken to check the stability of *Pashanbhedadi Churna* with respect to its microbial contamination at different climatic conditions and temperature setups at regular interval for a period of 11 months.

AIM

To study the stability of *Pashanbhedadi Churna* and to check microbial contamination in the *Pashanbhedadi Churna* at different time interval: at different climatic conditions, humidity and temperature set ups.

MATERIALS AND METHODS

Sample of *Pashanbhedadi Churna* was prepared (stored at room temperature) and finished product was studied for checking microbial contamination at regular intervals for a period of 1 year trial of the study completed. Microbiological study has been carried out in Microbiology Laboratory, ITRA., Jamnagar.

The first microbiological study was done on 5th day of drug preperation, Before giving it to the patients. Then samples from same container were given for the microbilogical study on random intervals during different seasons.

Drug

All the raw drugs were procured from Pharmacy of Gujarat Ayurved University, Jamnagar. The ingredients and the part used are given in (Table 1).

Table 1: Ingredients of *Pashanbhedadi Churna*[3]

S.No.	Drug	Botanical name	Part used	Quantity
1	*Pashanbhed*	*Bergenia ligulata* (Wall.)Engl.	*Mula*	1 part
2	*Vasa*	*Adhatoda vasica* Nees	*Patra*	1 part
3	*Gokshura*	*Tribulus terrestris* Linn.	*Mula*	1part
4	*Patha*	*Cissampelos paeria* Linn.	*Phala*	1part
5	*Haritaki*	*Terminalia chebula* Retz	*Phala*	1part
6	*Sunthi*	*Zinziber offcinale* Roscoe	Rhizomes	1/3part
7	*Maricha*	*Piper nigrum* Linn	*Phala*	1/3part
8	*Pippali*	*Piper longum* Linn	*Phala*	1/3part
9	*Shati*	*Hedychium spicatum* Ham	*Mula*	1part
10	*Dantimoola*	*Baliospermum montanum* Muell	*Mula*	1part
11	*Ajwain*	*Trachyspermum ammi* Sprague Linn	*Beeja*	1part
12	*Utkunchika*	*Centratherum anthelminctium* Kuntze	*Phala*	1part
13	*Hingu*	*Ferula narthex* Boiss	*Satva*	1part
14	*Bruhati*	*Solanum indicum* Linn	*Mula*	1part
15	*Kantakari*	*Solanum surattense* Burm	*Mula*	1part
16	*Vacha*	*Acorus calamus* Linn	*Mula*	1part
17	*Trapushabeeja*	*Cucumis sativus* Linn	*Beeja*	1part

Date of Drug Preparation: 19/02/2021

Storage

Pashanbhedadi Churna was stored in air-tight plastic containers, in the open light area and at room temperature in the department. Dry and clean stainless steel spoon was used for taking medicine.

Microbial Contamination

Microbial contamination was assessed by two methods to check any mycological findings and bacteriological findings.

1. **Smear Examination**
   A) Wet mount/10% K.O.H. Preparation
   B) Gram's stain
2. **Culture Study**
   A) Fungal culture
   B) Aerobic culture

The details of the procedures followed are given below.

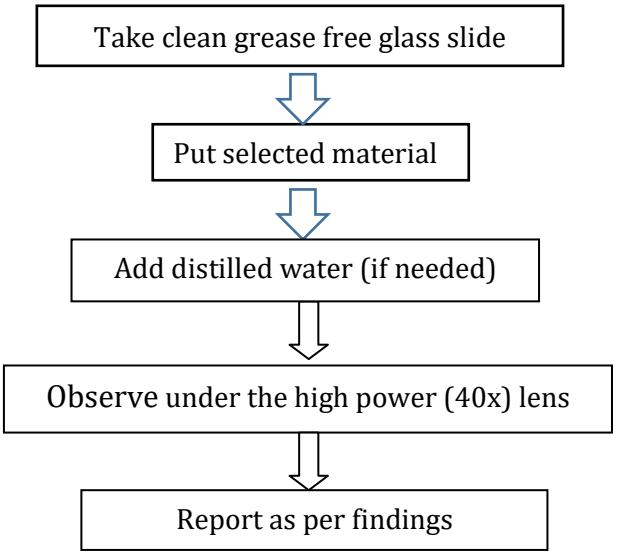
1. Smear Examination

A. Wet mount /10% K.O.H. Preparation

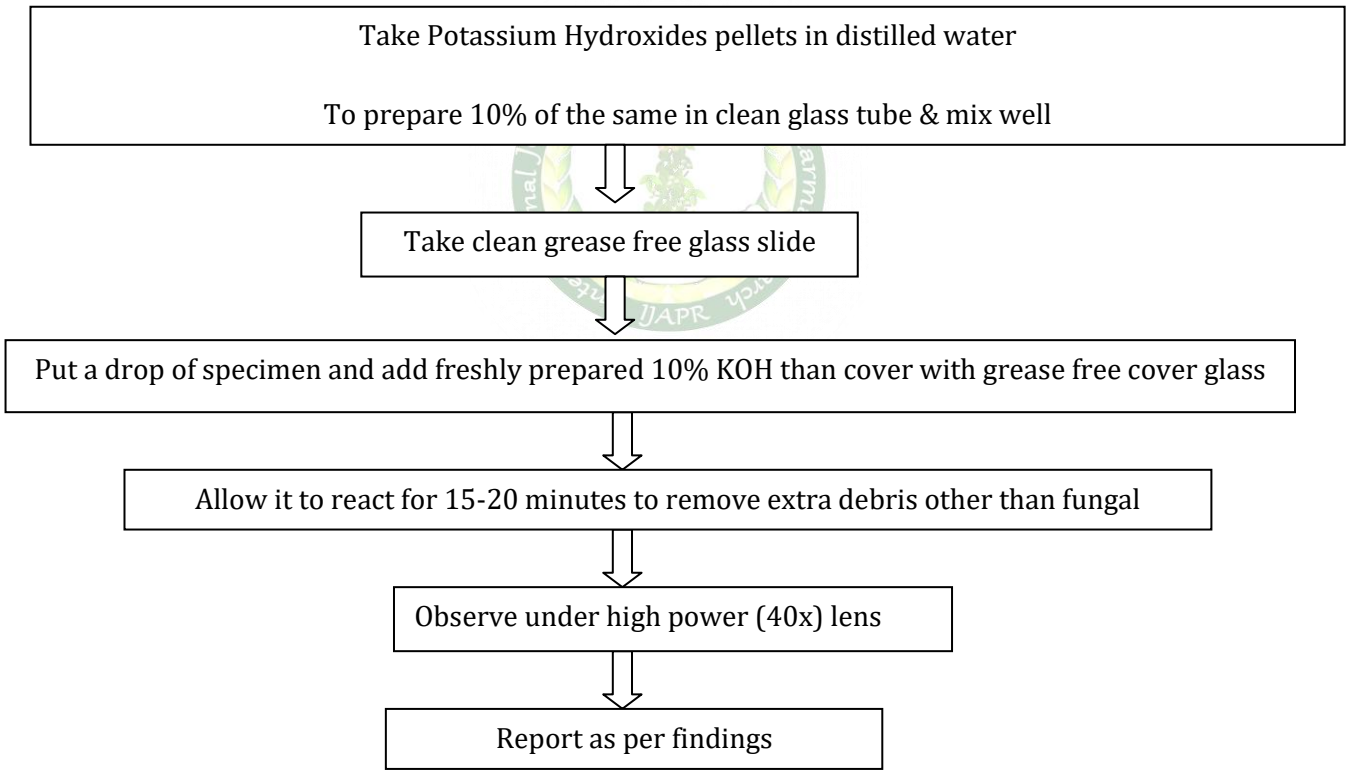
Aim: To rule out any mycological findings.

Specimen: ***Pashanbhedadi Churna***

Procedure for Wet Preparation



Procedure For 10% KOH Preparation



B. Gram's Stain Test

This test differentiates bacteria into two groups: gram positive and gram negative. The procedure is based on the ability of microorganisms to retain color of the stains used during the gram stain procedure. Gram negative bacteria are decolorized by any organic solvent (acetone or Gram's decolorizer) while Gram positive bacteria are not decolorized as primary dye retained by the cell and bacteria will remain as purple. After decolonization step, a counter stain effect found on Gram negative bacteria and bacteria will remain pink. This procedure allows bacteria to retain color of the stains, based on the differences in the chemical as well as physical properties of the cell wall (Alfred E Brown, 2001)[4]

Aim: To rule out any bacteriological findings.

Specimen: *Pashanbhedadi Churna*

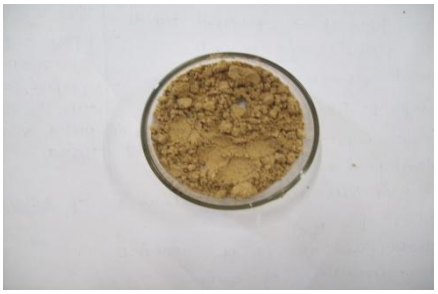


Figure 1: *Pashanbhedadi Churna*

Procedure for Gram's Stain

Take clean grease free glass slide to prepare dry equal thick preparation (i.e. smear)

↓

Fixed prepared smear by passing 3-4 times over the flame of Bunsen burner (The fixation kills vegetative form of microbes and render them permeable to stain, make material stick to the surface of slide & prevent autolytic changes)

↓

Cover fixed prepared smear with Gram's crystal violet solution and allow to Remain for mentioned time as per kit procedure

↓

Washed off smear to remove excessive reagent with tap water

↓

Cover smear with Gram's Iodine solution and allow remaining for mentioned time as per kit procedure

↓

Washed off smear to remove excessive reagent with tap water

↓

Decolorize smear with Gram's decolorizer by holding the slide at slope position and pour gram's decolorizer – acetone from its upper end up to removal of color of primary dye (i.e. Gram's Crystal Violet) or as per kit procedure

↓

Washed off smear to remove excess acetone with tap water

↓

Cover smear with Safranin solution and allow remaining for mentioned time as per kit procedure

↓

Washed off smear to remove excessive reagent with tap water

↓

Blot and allow to dry smear

↓

Examine under oil immersion lens and report as per findings

Culture Study

a. Fungal Culture Method

Following materials were collected with sterile cotton swab for inoculation purpose on selected fungal culture media (i.e. an artificial preparation).

Name of media: Sabouraud Dextrose Agar Base (SDA),
Modified (Dextrose Agar Base, Emmons)

Use of media: For selective cultivation of pathogenic fungi.

Company: HIMEDIA Laboratories Pvt. Ltd.

Required temperature: 37°C

Required time duration: 05 to 07 days

Procedure for Fungal Culture

In the clinical microbiology laboratory culture method are employed for isolation of organisms (The lawn/streak culture method is routinely employed)

↓

Choose appropriate selective solid media for inoculation purpose

↓

Dry selective solid media in Hot Air Oven before specimen inoculation
Allow to cool dried medium before Specimen inoculation

↓

Inoculate selective specimen by sterile cotton swab or by Nichrome wire (24 S.W.G. size) loop [First sterile loop in Bunsen burner oxidase flame-blue flame and allow it cool after that loop is charged by selected specimen to be cultured. One loopful of the specimen is transferred onto the onto the surface of well dried culture media]

↓

After inoculation / streaking process incubate inoculated medium in inverted position at 37°C for 05 to 07 to 21 days in incubator (incubation days are as per growth requirement) under aerobic atmosphere

↓

After selected incubation period examined growth by naked eye in form of colony or areal growth and confirm growth by performing different related biochemical reactions and different related staining procedures. After that report isolates

b. Aerobic Culture Method

Following materials were collected with sterile cotton swab for inoculation purpose on selected aerobic culture media (i.e. an artificial preparation)

Name of media: MacConkey Agar (MA) and Columbia Blood agar (BA)

Company: HIMEDIA Laboratories Pvt. Ltd.

Required time duration: 24 to 48 hours

Required temperature: 37°C

Use of media: for selective cultivation of pathogenic bacteria.

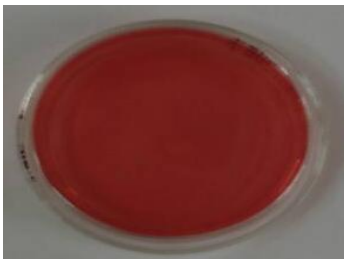


Figure 2: Mac Conkey Agar (MA)

Procedure for Aerobic Culture

In the clinical microbiology laboratory culture method are employed for isolation of organism (The streak culture method is routinely employed)

↓

Choose appropriate selective solid media for inoculation purpose

↓

Dry selective solid media in Hot Air Oven before specimen inoculation, allow to cool dried medium before specimen inoculation

↓

Inoculate selected specimen by four flame method (the loop should be flamed and cooled between the different sets of streaks i.e. four time) on surface of cool dried medium with nichrome wire (24 S.W.G. size) loop [first sterile loop in Bunsen burner oxidase flame –blue flame and allow it to cool than loop is charged with selected specimen to be cultured. One loopful of the specimen is transferred onto the surface of well dried plate]

↓

After streaking process incubate inoculated medium in inverted position at 37°C for 18-24 hours in incubator under aerobic or 10% CO_2 atmosphere

↓

After selected incubation period examined growth by naked eye in form of colony and confirm growth by performing different related biochemical reactions and different related staining procedures. After that report isolates

OBSERVATIONS AND RESULTS

Every time sample (in which drug preserved) were subjected to the microbiological study from the date of the preperation to the date of last microbiological study.

Table 2: Showing observations of sample preserved at room temperature

S.No.	Days of investigations After preparation of the sample	Temp.	Humidity	Observations of sample			
				Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
1.	5 Days	31°C	27%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
2.	63 Days	37°C	23%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
3.	89 Days	38°C	44%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
4.	191 Days	32°C	74%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
5.	282 Days	30°C	35%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
6.	315 Days	22°C	41%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated

This trial drug, *Pashanbhedadi Churna* was used in the treatment of *Ashmari* specifically in renal stone for the research work at ITRA and this drug has shown good result in *Ashmari*. So, the present Study was carried out to observe the stability study of *Pashanbhedadi Churna* with respect to Microbial Contamination of sample prepared and preserved in different climatic and temperature conditions. Thus a baseline Microbial profile was studied for approx. 11 months. At the end of study, it was found that there was no any microbial contamination was found in the preserved drug sample.

Stability is usually expressed in term of shelf-life, it is the time period from the drug production until the time it is intended to be consumed. Microorganism needs temperature, water and humidity at suitable environmental to develop and multiply. At different time intervals with humidity and temperature variation drug stability carried out.

DISCUSSION

Several factors are used to determine a product's shelf-life, ranging from organoleptic qualities to microbiological study. So, microbiological study of the *Pashanbhedadi Churna* showed the quality of *Churna* is in a standard condition. There were no any growth found of microorganisms neither bacterial nor fungal, till 11/01/2022 i.e., approximately 11 months from the date of preparation, which shows its good shelf life.

CONCLUSION

So, it is concluded that drug is stable in minimum 32°C temperature to maximum 38°C and minimum humidity 23% to maximum 74% humidity. That means stability duration of drug after preparation is approximately 11 months.

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