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Mishra Gauridutt
Ph.D. Scholar, Department of Roga Nidana Evam Vikriti Vijnana, Institute for Post Graduate Teaching & Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India

Cholera MS
Head, Microbiology Laboratory, Institute for Post Graduate Teaching & Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India

Pandya DH
Assistant Prof. Department of Roga Nidana Evam Vikriti Vijnana, Institute for Post Graduate Teaching & Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India

Correspondence
Mishra Gauridutt
Ph.D. Scholar, Department of Roga Nidana Evam Vikriti Vijnana Institute for Post Graduate Teaching & Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India

Stability study of coarse powder of Shunthi (*Zingiber officinale*), used in treatment of Sama stage (Acute stage) of *Amavata* (*Rheumatoid arthritis*) along with castor oil - with respect to baseline microbial diagnostic modalities

Mishra Gauridutt, Cholera MS and Pandya DH

Abstract
Background: In the disease *Amavata* the major complaints for which patients seeks medical supervision are moderate to severe pain, swelling, tenderness and morning stiffness in affected joints which restrict joints movement. *Amavata* is correlated with Rheumatoid arthritis (RA) due to same presentation of symptoms. The symptoms mention here are characteristic of *Ama* & without treating *Ama* it's not possible to treat the disease.
Aims: To carried out study for coarse powder of *Shunthi* (*Zingiber officinale*) with respect to its stability against microbial contamination.
Materials and Methods: Sample of Coarse powder of *Shunthi* was prepared and studied to check microbial contamination at regular time intervals.
Results: Every time sample was subjected to the microbiological study from the date of the preparation to the date of last microbiological study. No any contaminations was found in microbiological study.
Discussion: Hence the present Study was carried out to observe the stability study of coarse powder of *Shunthi* with respect to Microbial Contamination of sample prepared and store in different climatic conditions and temperature. Thus a baseline Microbial profile was studied at regular interval of 1 month for total 1 year & 1 month (i.e. time for consumption of prepared drug). At the end of study it was found that sample was not showed presence of any Microbes.
Conclusion: In microbiological study of the coarse powder of *Shunthi* there were no growth found of microorganisms (bacterial or fungal), till 13th Feb 2016 i.e. 01 year & 01 month from the date of preparation, shows its stability and good shelf life. Hence in present study the stability test of Coarse Powder of *Shunthi* with respect to microbiological findings was negative at room temperature, warm and cold, dry and humid conditions.

Keywords: *Amavata*, rheumatoid arthritis, RA, coarse, *Shunthi*, microbial contamination

Introduction
Amavata is made up of two words, *Ama* & *Vata*. *Ama* means incomplete digestion of food which result in incomplete/improper formation of *Annarasa*, circulate in body & reach to target cell where it produces pathology like heaviness in body, loss of strength, drowsiness, aggravation of *Vata* & improper elimination of waste product. Body ache, not desire to take food, thirst, fever, incomplete digestion of food, swelling in affected joints are the symptoms of *Amavata*. [1] When disease grow in intensity it become difficult to cure. All symptoms mention are characteristic of *Ama* & without treating *Ama* it is not possible to treat the disease so in this condition drug having *Ushna*, *Tikshna*, *Deepan*, *Pachan*, *Vatashamak*, *Shothhara* Properties can be used, when *Ama* is digested then drug having *Vatashamak* properties remain useful, only after digestion of *Ama* other drugs can be used. The property of *Ama* is *Guru*, *Snigdha*, *Sheet*, *Pichchhil* and *Manda*. In Ayurvedic classics *Erand Sneha* has been mentioned as drug of choice for the disease *Amavata* [2] due to having its *Katu*, *Tikshna*, *Ushna* properties [3] *and Shunthi* increase digestive fire, make interest toward food and pacify *Vata* and *Kapha*. The drug was prepared in pharmacy of Gujarat Ayurved University, Jamnagar. No any preservative was added to the test drug. Drug preparation was finished on 11 January 2016. Finished product was stored in airtight plastic container at room temperature. Thus in the present study on attempt was taken to check stability of Coarse powder with respect to its Microbial profile at different climatic conditions and temperature setups at regular interval for a period of 1 year 1 month.

Aim
To study the stability of finished product and to check microbial contamination in the finished product at different time interval- at different climatic conditions, temperature and humidity set ups.

Material and Method
Sample of coarse powder of *Shunthi* was prepared (stored at room temperature) and finished product studied to check microbial contamination at regular intervals for a period of 1 year 1months (upto drug used). Microbiological study has been carried out in Microbiology Laboratory, I. P. G. T. & R. A., Jamnagar. Mainly 02 studies have been carried out to rule out that presence of any bacteria or fungi in the prepared drug as a final finished product. The initial microbiological study was done on 30th day of preparation, before giving drug to the patients. Then sample from same bag was subjected to the micro bilogical study regularly with random intervals during different seasons.

Drug material
The drug was obtained from Pharmacy of Gujarat Ayurved University, Jamnagar.

Date of Drug Preparation: 11 January 2016

Storage
Finished product of coarse powder of *shunthi* was stored in air-tight food grade, plastic containers, stored in the open light area in the department at room temperature. Clean and dry stainless steel spoon was used to take medicine.

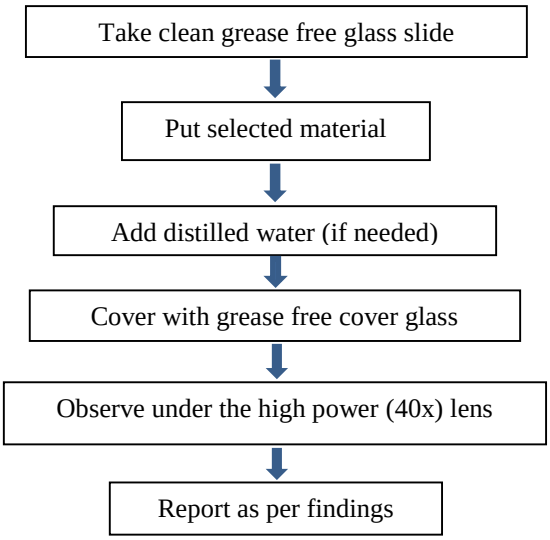
Microbial profile
Microbial contamination was assessed by two methods to check any mycological findings and bacteriological findings.

1. Smear Examination
A. Wet mount /10% K.O.H. Preparation
B. Gram's stain

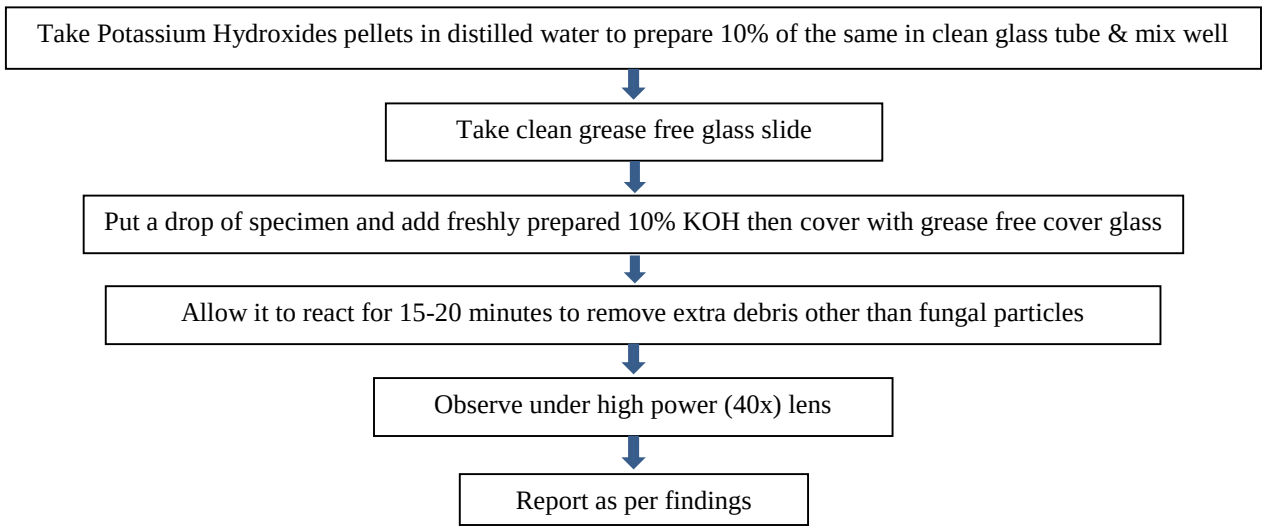
2. Culture Study
A. Fungal culture
B. Aerobic culture
The details of the procedures followed are given below.

1. Smear Examination
A. Wet mount /10% K.O.H. Preparation
Aim: To rule out any mycological findings.
Specimen: Coarse powder of *shunthi*

Procedure for wet preparation



Procedure For 10% KOH Preparation



B. Gram's stain test

Gram staining is a differential staining technique that 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 decolorization step, a counter stain effect found on Gram negative bacteria and bacteria will remain pink. The Gram stain procedure enables bacteria to retain color of the stains, based on the differences in the chemical and physical properties of the cell wall (Alfred E Brown, 2001) [4].

Aim: To rule out any bacteriological findings.

Specimen: Coarse powder of *Shunthi*

Procedure for Gram's Stain

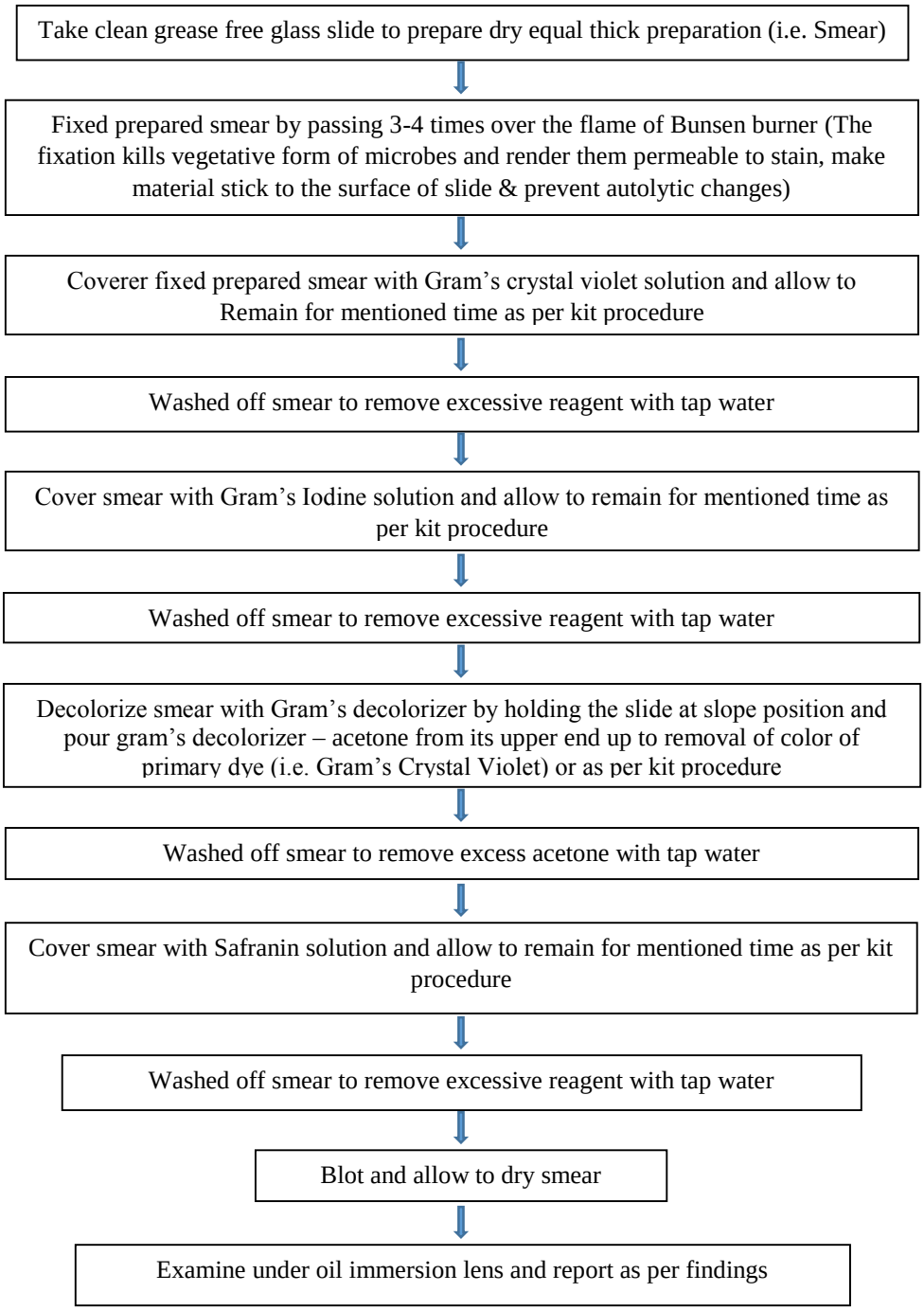



Fig 1, 2: Smear staining procedure

A. Fungal culture method:
Respected materials 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)

Company: HIMEDIA Laboratories Pvt. Ltd.

Required time duration: 05 to 07 days

Required temperature: 37 ºC

Use of media: For selective cultivation of pathogenic fungi.

Fig 3: Sabouraud Dextrose Agar Base (SDA) Bottle

Procedure for Fungal Culture

In the clinical microbiology laboratory culture method are employed for isolation of organism (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

↓

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 than loop is charged with selected specimen to be cultured. One loopful of the specimen is transferrd onto the onto the surface of well dried culture media]

↓

After inoculation/ streaking process incubate inoculated medium in inverted position at 37 ^{0}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 Arial growth and confirm growth by performing different related biochemical reactions and different related staining procedures. After that report is isolates.

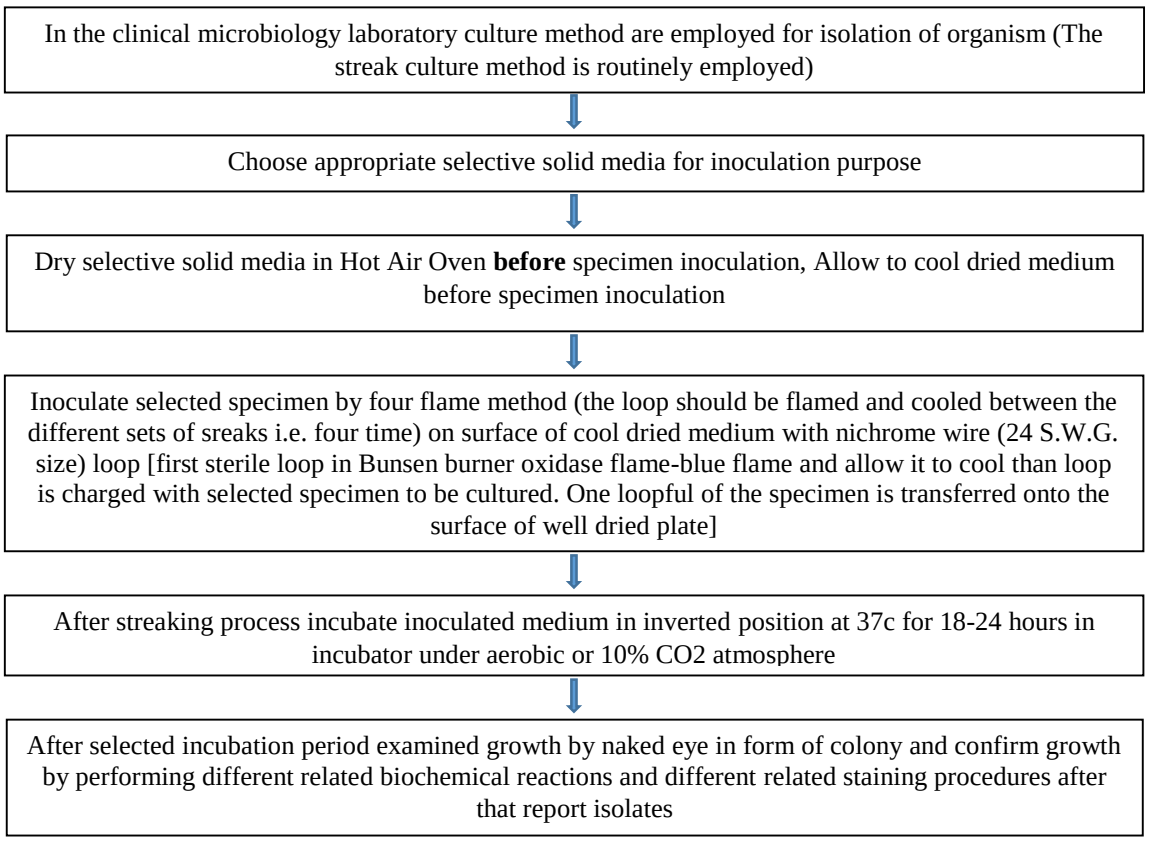
Aerobic culture method
Respected materials collected with sterile cotton swab for inoculation purpose on selected aerobic culture media (i.e. an artificial preparation)

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

Company: HIMEDIA Laboratories Pvt. Ltd.

Required time duration: 24 to 48 hours
Required temperature: 37 ºC
Use of media: For selective cultivation of pathogenic bacteria.

Procedure for Aerobic Culture



Observations and Results
Every time samples were subjected to the microbiological study to rule out stability of prepared drug up to completion of the same.
Results are shown in table no 1.

Table 1: Showing observations of samples preserved at room temperature

Sr. No.	Days of study at	Temp & Humidity	Date of sample given	Observations of sample			
				Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
1.	30th Day	24 ^{0}C, 33%	10th Feb 2016	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
2.	60th Day	31 ^{0}C, 39%	10th March 2016	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
3.	92nd Day	34 ^{0}C, 38%	12th April 2016	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
4.	135th Day	36 ^{0}C, 44%	25th May 2016	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
5.	144th Day	34 ^{0}C, 55%	13th June 2916	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
6.	170th Day	30 ^{0}C, 72%	9th July 2016	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
7.	201st Day	27 ^{0}C, 89%	10th Aug 2016	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
8.	236th Day	34 ^{0}C, 55%	15th Sept 2016	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
9.	264th Day	29 ^{0}C, 58%	13th Oct 2016	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
10.	302nd Day	30 ^{0}C, 31%	21st Nov 2016	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated

11.	324th Day	27^{0}C, 45%	13th Dec 2016	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
12.	374th Day	27^{0}C, 45%	23rd Jan 2017	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
13.	384th Day	27^{0}C, 38%	13th Feb 2017	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated

Discussion

For better safety and efficacy, drug should be free from any type of microbial contamination. Stability of drug is expressed in term of its Shelf life. The factors affecting stability of prepared drug are categorized under intrinsic and extrinsic factor (FDA report 2001). Intrinsic factors include moisture content, acidity, nutrient content, biological structure, redox potential, naturally occurring and added antimicrobials. Extrinsic factors include types of packaging, effect of time/temperature on microbial growth, storage/holding conditions and processing steps (FDA report 2001). Microbial contamination should avoided to increase drug stability and storage time. Coarse powder of *Shunthi* was prepared and stored at room temperature. Sample was selected randomly for study of microbiological contamination. Changes in temperature and humidity of environment was observed during study period. Optimum temperature for microbial growth is temperature at which microbes multiplies, this optimum temperature for psychrophilic bacteria (low temperate loving) is -20 to +10 ^{0}C while for mesophilic bacteria (moderate temperate loving) and thermophilic (high temperate loving) bacteria is 20-45 ^{0}C and 41-122 ^{0}C respectively. The region where the drug was prepared and sample was stored was very proximal to sea coast, this area has longest sea shore and maximum number of sea ports, so relative humidity (RH) remains high in all the seasons of the year. Highest RH observed was 89% in month of August while lowest relative humidity was 31% observed in month of November (as shown in Table 1). High RH may allow the growth of microbes [5], RH remain variable during whole study period, although air cannot be considered dry at RH more than 40%. Wet mount, fungal culture, gram stain and aerobic culture tests were used to rule out any fungal and bacterial contamination in the sample of monthly interval from 10th Feb 2016 to 13th Feb 2017. During this study period no any microbes were isolated as a result of aerobic culture and no any fungal pathogen were isolated as a result of fungal culture (as shown in Table 1). Moisture content of drug play important role in its long term storage. Moisture contents main causative factor in drug deterioration, it also act as an enzymatic activator which slowly decompose the drug resulting in its degradation [6]. In *Ayurvedic* classics *Shunthi* increase digestive fire, make interest toward food and pacify *Vata* and *Kapha*. In present study *Shunthi* has shown a good and promising result in *Amavata*. Coarse powder of *Shunthi* was used in decoction form along with castor oil in *Sama* stage (Acute stage) of *Amavata* (Rheumatoid arthritis).

Conclusion

Shelf- life is the time period from when the product is produced until the time it is planned to be consumed or used. Several factors are used to determine a product's shelf-life, ranging from organoleptic qualities to microbiological safety. Hence Microbiological study of the coarse powder of *Shunthi* showed that the quality of coarse powder is in a standard condition. There were no growth found of microorganisms (bacterial or fungal), till 13th Feb 2017 i.e. 01 year & 01 month from the date of preparation, shows its good shelf life.

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