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

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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 Anarasa, 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, Pichchihil 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 microbiological 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

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Take clean grease free glass slide

Put selected material

Add distilled water (if needed)

Cover with grease free cover glass

Observe under the high power (40x) lens

Report as per findings
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Procedure For 10% KOH Preparation

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Take Potassium Hydroxides pellets in distilled water to prepare 10% of the same in clean glass tube & mix well

Take clean grease free glass slide

Put a drop of specimen and add freshly prepared 10% KOH then cover with grease free cover glass

Allow it to react for 15-20 minutes to remove extra debris other than fungal particles

Observe under high power (40x) lens

Report as per findings
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**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*

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**Procedure for Gram’s Stain**

1. Take clean grease free glass slide to prepare dry equal thick preparation (i.e. Smear)
2. 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)
3. Cover fixed prepared smear with Gram’s crystal violet solution and allow to remain for mentioned time as per kit procedure
4. Washed off smear to remove excessive reagent with tap water
5. Cover smear with Gram’s Iodine solution and allow to remain for mentioned time as per kit procedure
6. Washed off smear to remove excessive reagent with tap water
7. 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
8. Washed off smear to remove excess acetone with tap water
9. Cover smear with Safranin solution and allow to remain for mentioned time as per kit procedure
10. Washed off smear to remove excessive reagent with tap water
11. Blot and allow to dry smear
12. Examine under oil immersion lens and report as per findings
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.

**Procedure for Fungal Culture**

1. In the clinical microbiology laboratory culture method are employed for isolation of organism (The lawn/streak culture method is routinely employed)
2. Choose appropriate selective solid media for inoculation purpose
3. Dry selective solid media in Hot Air Oven before specimen inoculation
4. 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 transferred onto the surface of well dried culture media]
5. After inoculation/ streaking process incubate inoculated medium in inverted position at 37 ºC for 05 to 07 to 21 days in incubator (incubation days are as per growth requirement) under aerobic atmosphere
6. After selected incubation period examined growth by naked eye in form of colony or Aerial 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 °C
Use of media: For selective cultivation of pathogenic bacteria.

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 sreaks 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 37c for 18-24 hours in incubator under aerobic or 10% CO2 atmosphere

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

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Days of study at</th>
<th>Temp &amp; Humidity</th>
<th>Date of sample given</th>
<th>Observations of sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Gram's Stain</td>
</tr>
<tr>
<td>1.</td>
<td>30th Day</td>
<td>24 °C, 33%</td>
<td>10th Feb 2016</td>
<td>Microorganisms not seen</td>
</tr>
<tr>
<td>2.</td>
<td>60th Day</td>
<td>31 °C, 39%</td>
<td>10th March 2016</td>
<td>Microorganisms not seen</td>
</tr>
<tr>
<td>3.</td>
<td>92nd Day</td>
<td>34 °C, 38%</td>
<td>12th April 2016</td>
<td>Microorganisms not seen</td>
</tr>
<tr>
<td>4.</td>
<td>135th Day</td>
<td>36 °C, 44%</td>
<td>25th May 2016</td>
<td>Microorganisms not seen</td>
</tr>
<tr>
<td>5.</td>
<td>144th Day</td>
<td>34 °C, 55%</td>
<td>13th June 2916</td>
<td>Microorganisms not seen</td>
</tr>
<tr>
<td>6.</td>
<td>170th Day</td>
<td>30 °C, 72%</td>
<td>9th July 2016</td>
<td>Microorganisms not seen</td>
</tr>
<tr>
<td>7.</td>
<td>201st Day</td>
<td>27 °C, 89%</td>
<td>10th Aug 2016</td>
<td>Microorganisms not seen</td>
</tr>
<tr>
<td>8.</td>
<td>236th Day</td>
<td>34 °C, 55%</td>
<td>15th Sept 2016</td>
<td>Microorganisms not seen</td>
</tr>
<tr>
<td>9.</td>
<td>264th Day</td>
<td>29 °C, 58%</td>
<td>13th Oct 2016</td>
<td>Microorganisms not seen</td>
</tr>
<tr>
<td>10.</td>
<td>302nd Day</td>
<td>30 °C, 31%</td>
<td>21st Nov 2016</td>
<td>Microorganisms not seen</td>
</tr>
</tbody>
</table>
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 °C while for mesophilic bacteria (moderate temperate loving) and thermophilic (high temperate loving) bacteria is 20-45 °C and 41-122 °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.

References