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# Evaluation of Baseline Microbial Stability of *Avipattikara Churna* used in the treatment of *Amlapitta*

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## Abstract

**Introduction**: The evolving lifestyle has given rise to various lifestyle disorders, *Amlapitta* is one of the gastrointestinal disorders presenting symptoms such as retrosternal burning, nausea, and vomiting among others. Current medications for hyperacidity include Antacids, H2 Blockers, Proton Pump Inhibitors among others. However, they have side effects such as H2 Blockers (diarrhea, constipation, fatigue, drowsiness, headache, and muscle aches).[i] PPI (headache, rash, dizziness, and gastrointestinal symptoms including nausea, abdominal pain, flatulence, constipation, and diarrhea.)[ii] *Avipattikara Churna* mentioned first in *Bhaishajya Ratnavali* is an effective drug in Ayurveda in treating *Amlapitta*.[iii] However, nearly half of its composition is *Sharkara* (sugar), which, being hygroscopic, absorbs moisture when exposed to humidity, potentially leading to fungal contamination. This compromises the shelf life of the formulation. Thus it is important to evaluate the baseline microbial stability of *Avipattikara Churna*. **Aim**: To evaluate the stability of *Avipattikara Churna* and monitor microbial contamination in the finished product over varying time intervals and under diverse climatic conditions, temperature, and humidity settings **Materials and methods**: Samples of *Avipattikara Churna* (stored at room temperature) were studied to inspect microbial contamination under different climatic conditions for 240 days The initial microbiological study was done on the 35$^{th}$ day of preparation, just before its oral administration to the patients. Then samples from same container were subjected to the microbiological study regularly with random intervals during different seasons for a period of 8 months. **Result**: In the present study, the stability study of *Avipattikara Churna* concerning microbiological findings was negative at room temperature, warm and cold, and dry and humid conditions for 240 days.

**Keywords:** *Avipattikara Churna,* Microbial profile, Stability study

## Introduction

The evolving lifestyle has given rise to various lifestyle disorders, *Amlapitta* is one of the gastrointestinal disorders presenting symptoms such as retrosternal burning, nausea, and vomiting among others. Current medications for hyperacidity include Antacids, H2 Blockers, Proton Pump Inhibitors among others. But they

have side effects such as H2 Blockers (diarrhea, constipation, fatigue, drowsiness, headache and muscle aches.)[iv] PPI (headache, rash, dizziness, and gastrointestinal symptoms including nausea, abdominal pain, flatulence, constipation, and diarrhea.)[v] *Avipattikara Churna* mentioned first in *Bhaishajya Ratnavali* is an effective drug in Ayurveda in treating *Amlapitta*.[vi] However, nearly half of its composition is *Sharkara* (sugar), which, being hygroscopic, absorbs moisture when exposed to humidity, potentially leading to fungal contamination. This compromises the shelf life of the formulation.

In recent decades, the market for herbal, herbo-mineral, and traditional medicines have grown exponentially. Despite this growth, the primary limitation in the widespread adoption of traditional medicines is the lack of data on their stability and shelf life. Stability research confirms how the nature of a medicinal substance or drug changes over time, influenced by environmental factors like temperature, humidity, and light. It also determines the drug's shelf life and recommended storage conditions. Therefore, stability studies are a fundamental aspect of evaluating drug quality.

Therefore, this study aims to examine the stability of *Avipattikara Churna*, focusing on its resistance to microbial contamination under various climatic conditions and temperatures. The drug in the present study was prepared in the Pharmacy of ITRA, Jamnagar under all possible hygienic conditions. No artificial preservative was added to the test drug Drug preparation was finished on the 5$^{th}$ of September 2023. The finished product was stored in an airtight plastic container at room temperature. In the present study, an attempt is made to check the stability of *Avipattikara Churna* concerning its microbial profile at different climatic conditions and temperature setups at regular intervals for 240 days.

**KEYWORDS**: *Avipattikara Churna*, Microbial profile, Stability study

**Aim**: To evaluate the stability of *Avipattikara Churna* and monitor microbial contamination in the finished product over varying time intervals and under diverse climatic conditions, temperature, and humidity settings, aiming to ensure adherence tostandardization protocols for study drug production.

**Materials and methods:** Samples of *Avipattikara Churna* (stored at room temperature) were studied to inspect microbial contamination under different climatic conditions for 240 days The study was conducted at the Microbiology Laboratory, Institute of Teaching and Research in Ayurveda (ITRA), Jamnagar, Gujarat, India. Mainly two tests were performed to rule out the existence of any bacteria or fungi in the finished product sample of the prepared drug. The initial microbiological study was done on the 35$^{th}$ day of preparation, just before its oral administration to the patients. Then samples from same container were subjected to the microbiological study regularly with random intervals during different seasons for a period of 8 months.

Date of Drug Preparation : 05/09/2023

Storage : Finished product of *Avipattikara Churna* was kept in air tight plastic container placed in the open light area at room temperature. Clean and dry stainless steel spoon was used to pack the medicine into small plastic bag.

**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**

➢ **PROCEDURE FOR 10% KOH PREPARATION:**

Take Potassium Hydroxides pellets (of HiMedia Lab. Pvt. Ltd.) 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 it with grease free cover glass

⬇

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

⬇

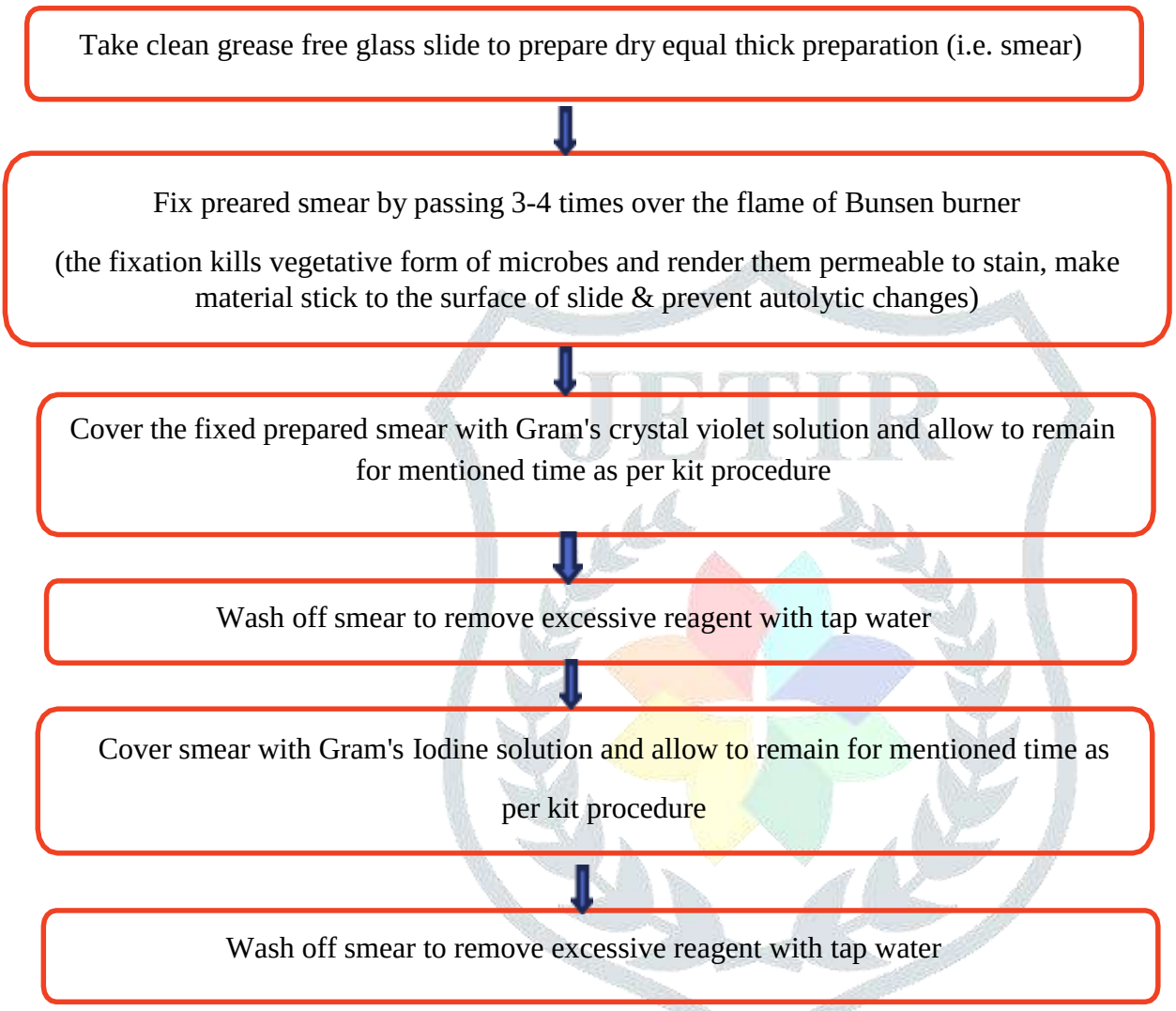
Observe under high power (40x) microscopic lens

⬇

Report as per finding

**B. Gram's stain**

➢ Procedure for Gram's Stain Test:



**Procedure for Gram's Stain**
**2. Culture Study**

**A. Fungal culture method**

Respected materials were collected with sterile cotton swabs for inoculation purposes 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 time duration: 05 to 07 days
Required temperature: 37 ºC

**Figure 1. Sabouraud Dextrose Agar Base (SDA)**

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

↓

Choose appropriate selective solid media for inoculation purpose

↓

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

↓

Wash off smear to remove excess acetone with tap water

↓

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

↓

Washed off smear to remove excessive reagent with tap water

↓

Procedure for Fungal Culture:

↓

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

↓

After inoculation / streaking process Incubate inoculated medium in inverted position at 37°Cfor 05 to 07 to 21-days in Incubator (incubation days are as per growth requirement) under aerobic atmosphere

↓

Inoculate selected *Avipattikara Churna* by Sterile cotton swab or by Nichrome wire (24 5.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 culture media]

↓

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 differentrelated staining procedures. After that report isolates.

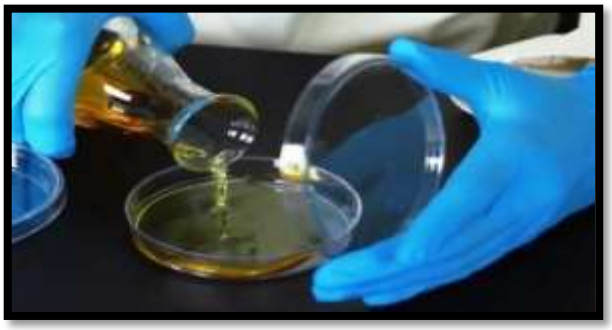


**Figure 2: Procedure for Fungal culture**

**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 : MacConkey 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.

**Figure 3: Mac Conkey Agar (MA)**

Procedure for Aerobic Culture: -

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

↓

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

↓

Inoculate selected *Avipattikara Churna* 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 culture. Oneloopful of the specimen was transferred onto the surface of well dried plate]

↓

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

↓

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

**Figure 4: Procedure for Aerobic culture**

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

**Observations and results**

Sample of *Avipattikara Churn*a which was stored at room temperature did not show presence of any mycological and bacteriological contamination on wet mount test, fungal culture, gram stain and aerobic culture test at the end of the study period. Results are shown in table below.

**Table: 1 High and low weather summary of temperature in Jamnagar, Gujarat during the study period.[vii]**

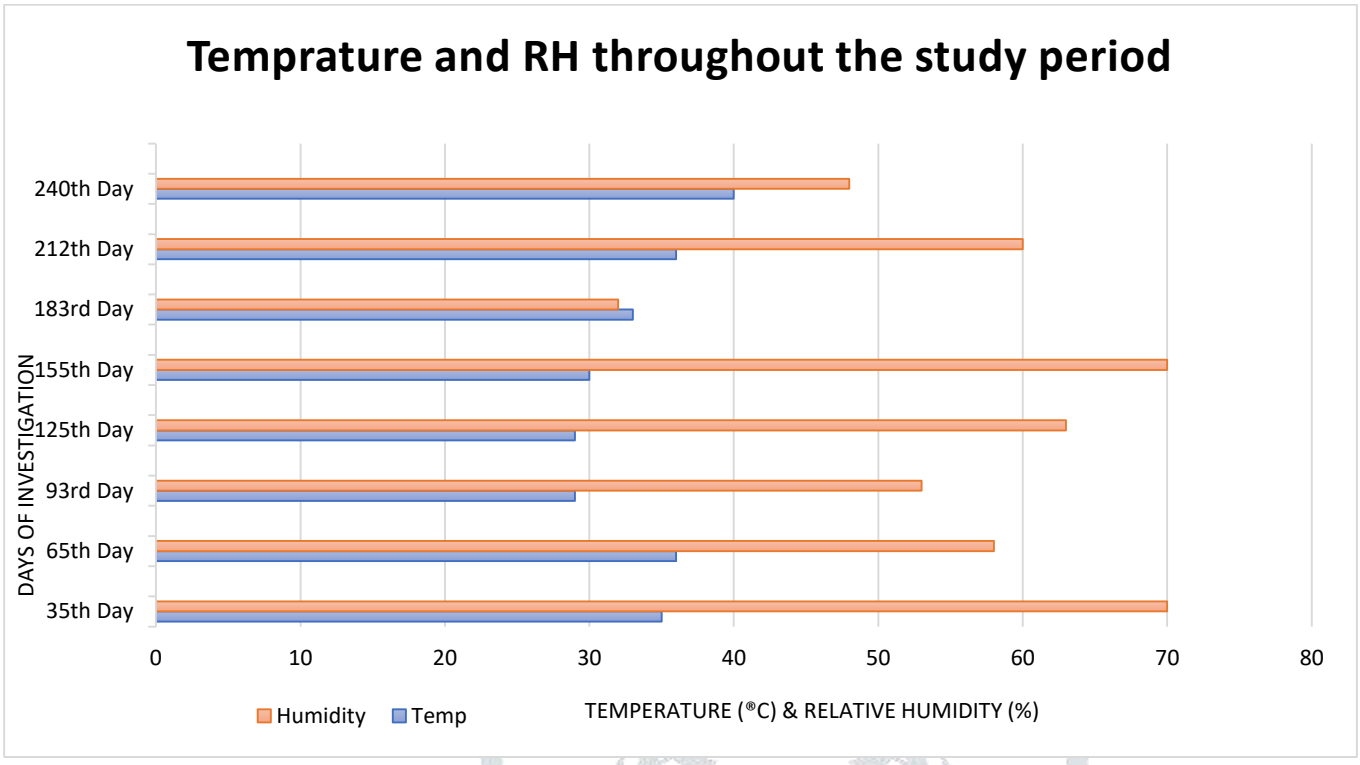
| Time period | Temperature (05 September 2023 to 02 May 2024) | | | | |
|---|---|---|---|---|---|
| | High | Date & time | Low | Date & time | Average |
| September | 40 °C | 15 Sep 2023; 14:30 | 25 °C | 18 Sep 2023; 05:30 | 29°C |
| October | 38 °C | 26 Oct 2023; 14:30 | 23 °C | 30 Oct 2023; 05:30 | 31°C |
| November | 37 °C | 11 Nov 2023; 14:30 | 18 °C | 29 Nov 2023; 05:30 | 27°C |
| December | 33°C | 24 Dec 2023; 14:30 | 15 °C | 12 Dec 2023; 08:30 | 23°C |
| January | 33°C | 12 Jan 2024; 14:30 | 13 °C | 4 Jan 2024; 05:45 | 22°C |
| February | 38°C | 29 Feb 2024; 17:30 | 13 °C | 10 Feb 2024; 05:30 | 25°C |
| March | 40 °C | 21 Mar 2024; 14:30 | 13 °C | 4 Mar 2024; 05:30 | 28°C |
| April | 41 °C | 9 Apr 2024; 17:30 | 21 °C | 5 Apr 2024; 20:30 | 31°C |
| May | 40 °C | 1 May 2024; 05:30 | 24 °C | 2 May 2024; 05:30 | 34°C |

**Table: 2 High and low weather summaries of relative humidity in Jamnagar, Gujarat during the study period.[viii]**

| Time period | Humidity (05 September 2023 to 02 May 2024) | | | | |
|---|---|---|---|---|---|
| | High | Date & time | Low | Date & time | Average |
| September | 100 % | 19 Sep 2023; 14:30 | 10% | 21 Sep 2023; 05:30 | 74% |
| October | 96% | 9 Oct 2023; 02:30 | 22% | 27 Oct 2023; 14:30 | 61% |
| November | 98% | 13 Nov 2023; 05:30 | 26% | 28 Nov 2023; 14:30 | 56% |
| December | 92% | 01 Dec 2023; 05:30 | 20% | 17 Dec 2023; 14:30 | 59% |
| January | 96% | 31 Jan 2024; 08:30 | 17% | 21 Jan 2024; 14:30 | 53% |
| February | 96% | 2 Feb 2024; 08:30 | 08% | 25 Feb 2024; 14:30 | 45% |
| March | 93% | 2 Mar 2024; 05:30 | 09% | 5 Mar 2024; 14:30 | 40% |
| April | 88% | 1 Apr 2024; 05:30 | 11% | 28 Apr 2024; 14:30 | 45% |
| May | 91% | 1 May 2024; 05:30 | 20% | 2 May 2024; 14:30 | 56% |

**Table: 3 Observation of *Avipattikara Churna* preserved at room temperature after preparation [ix](**Date of Drug Preparation: 05/09/2023)

| S.N. | Date & Days of investigations After preparation of the sample | Temperature (˚C) | Humidity (%) | Observation of samples | | | |
|---|---|---|---|---|---|---|---|
| | | | | Gram's Stain | Aerobic culture | Wet mount/ 10% KOH Preparation | Fungal culture |
| 1 | 10.10.2023<br>35th Day | 35 ˚C | 71% | Microorganisms Not Seen | No organisms isolated | Fungal filaments not seen | No Fungal Pathogen Isolated |
| 2 | 09.11.2023<br>65th Day | 36 ˚C | 58% | Microorganisms Not Seen | No organisms isolated | Fungal filaments not seen | No Fungal Pathogen Isolated |
| 3 | 07.12.2023<br>93rd Day | 29 ˚C | 53% | Microorganisms Not Seen | No organisms isolated | Fungal filaments not seen | No Fungal Pathogen Isolated |
| 4 | 08.01.2024<br>125th Day | 28 ˚C | 63% | Microorganisms Not Seen | No organisms isolated | Fungal filaments not seen | No Fungal Pathogen Isolated |
| 5 | 07.02.2024<br>155th Day | 30 ˚C | 70% | Microorganisms Not Seen | No organisms isolated | Fungal filaments not seen | No Fungal Pathogen Isolated |
| 6 | 06.03.2024<br>183rd Day | 33 ˚C | 32% | Microorganisms Not Seen | No organisms isolated | Fungal filaments not seen | No Fungal Pathogen Isolated |
| 7 | 04.04.2024<br>212th Day | 36 ˚C | 60% | Microorganisms Not Seen | No organisms isolated | Fungal filaments not seen | No Fungal Pathogen Isolated |
| 8 | 02.05.2024<br>240th Day | 40 ˚C | 48% | Microorganisms Not Seen | No organisms isolated | Fungal filaments not seen | No Fungal Pathogen Isolated |




**Discussion:**

Stability is usually expressed in terms of shelf life. The factors that may be considered when determining whether a prepared product requires time/temperature control during storage, distribution, sale, and handling may be categorized under intrinsic, extrinsic, and other factors. Intrinsic factors include moisture content, pH and acidity, nutrient content, biological structure, redox potential, and naturally occurring and added antimicrobials. Extrinsic factors include types of packaging/atmospheres, the effect of time/temperature conditions on microbial growth, storage/holding conditions, and processing steps. Microbial growth should be avoided to increase its stability period and the drug can be stored normally.

*Avipattikara Churna* was prepared and stored at room temperature. Sample was selected randomly for the study of microbial contamination. In this study highest and lowest temperatures observed at Jamnagar (study area) was 41°C in the months of April 2024 and 13°C in the months of January, February and March 2024 as shown in the table-1. Average temperature during the whole study period was 27.38 °C. The optimum temperature for bacterial growth is the temperature at which bacteria multiply. This optimum temperature for psychrophilic bacteria (cold-loving bacteria) is 15-20°C while for mesophilic (middle living) and thermophilic (heat-loving) bacteria it is 30-37°C and 50-60°C respectively. The optimum temperature for psychrophilic and mesophilic bacteria falls in the range of temperatures observed during the period of study.

The drug was prepared and stored in a coastal region with the state's longest seashore and most seaports. Consequently, relative humidity (RH) remains high throughout the year. Highest RH observed was 100% in the month of September while lowest humidity was 08% noted in month of February as shown in table-2. High humidity can allow the growth of microbes.[x] Relative humidity remained constantly high during the study duration.

Based on the days of investigation, highest temperature was observed on 2$^{nd}$ May 2024, and lowest temperature was noted on 8$^{th}$ Jan 2024. Maximum relative humidity was recorded at 71% on 10$^{th}$ October 2024 and minimum relative humidity was recorded at 32% on 6$^{th}$ March 2024 10% K.O.H preparation, Fungal culture, Gram stain, and Aerobic culture tests were used to rule out any fungal and bacterial contamination in the samples on approx. regular days interval from 05 September 2023 to 2 May 2024 i.e. for 240 days. During the study period, no organisms were isolated in aerobic culture, and no fungal pathogens were detected in the fungal culture as shown in table-3. Sample which was stored in condition open to all climatic changes did not

show any kind of microbial contamination. These results may contribute to some properties of formulation. Moisture content of formulation plays the most important role.

## Conclusion

The microbiological study of *Avipattikara Churna* confirms its preparation and storage under standard conditions, exhibiting no microbial growth, bacterial or fungal, for a remarkable eight-month period, highlighting its impressive shelf life.

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**Conflicts of interest**: There are no conflicts of interest

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