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A Stability Study for Usheeradi Taila for Prathama Patalagata Timira (Myopia): By Usage of Basic Diagnostic Modes

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ABSTRACT

Prathama Patalagata Timira is an important ocular disorder described in the Ayurvedic classics and is characterized by disturbances in visual perception, particularly difficulty in distant vision. Based on its comparable clinical features, Prathama Patalagata Timira [1] may be clinically correlated with Myopia, a common refractive error in which distant objects appear blurred due to the formation of the image in front of the retina. Despite the availability of corrective measures in contemporary ophthalmology, myopia continues to be an important cause of visual impairment. Ayurveda describes various therapeutic approaches for ocular disorders, including Kriyakalpa, Snehana, Tarpana, Nasya, and other measures. Usheeradi Taila, owing to its Ayurvedic therapeutic properties, may be considered in the management of Timira. Evaluation of the quality and stability of any medicinal formulation is essential for ensuring its safety and therapeutic utility. Among the various quality-control parameters, microbiological stability is particularly important to detect possible microbial contamination during storage.

Materials and Methods: The present study was undertaken to evaluate the microbiological stability of Usheeradi Taila prepared for the management of Prathama Patalagata Timira with clinical correlation to Myopia. The finished formulation was stored in suitable containers under different environmental conditions, including room temperature, warm, cold, dry, and humid conditions. The study was conducted for a period of eight months. Samples were collected at predetermined intervals and subjected to microbiological evaluation using **smear examination and culture methods** to assess the presence or absence of microbial contamination.

Results: Throughout the eight-month study period, Usheeradi Taila showed **no detectable microbial growth**. Smear examination and culture studies remained negative at all assessed intervals under the different storage conditions. No bacterial or other microbial contamination was observed.

Conclusion: The present study indicates that Usheeradi Taila maintained satisfactory **microbiological stability for eight months** under the tested environmental conditions. The absence of detectable microbial growth suggests that the formulation remained microbiologically stable during the period of observation.

KEYWORDS: Prathama Patalagata Timira, Myopia, Usheeradi Taila, Microbial Profile, Microbiological Stability, Stability Study, Ocular Disorder

INTRODUCTION-

Prathama Patalagata Timira is an important ocular condition described in the Ayurvedic classics and is characterized by disturbances in visual perception, particularly difficulty in seeing distant objects. Based on its clinical features, Prathama Patalagata Timira may be clinically correlated with Myopia, a common refractive error in which distant objects appear blurred due to the formation of the image in front of the retina. Myopia is influenced by various genetic and environmental factors and is increasingly recognized as an important cause of visual impairment. Ayurveda describes various approaches for the management of ocular disorders through Kriyakalpa, Snehan, Tarpana, Nasya, and other therapeutic measures. Usheeradi Taila is an Ayurvedic medicated oil that may be considered in the management of Timira based on its therapeutic properties. Proper evaluation of the quality and stability of the finished formulation is essential to ensure its safety, consistency, and therapeutic utility. Microbiological analysis is an important quality-control parameter for pharmaceutical preparations. It helps to determine the presence or absence of viable microorganisms and designated microbial contaminants. The stability and shelf-life of a formulation can be influenced by environmental factors such as temperature and relative humidity. Variations in these conditions during storage may promote microbial proliferation and consequently affect the quality and safety of the finished product. Therefore, appropriate evaluation under different climatic conditions is necessary to establish the microbiological stability of the formulation. Assessment of Usheeradi Taila at different time intervals and under varying temperature and humidity conditions can provide valuable information regarding its microbiological stability and storage suitability. Such evaluation contributes to standardization and quality assurance of the formulation intended for use in Prathama Patalagata Timira with clinical correlation to myopia.

AIM-To comprehensively assess the **microbiological stability of Usheeradi Taila** as a finished product and to monitor microbial contamination at different time intervals under varying climatic conditions and fluctuations in temperature and humidity.

OBJECTIVE

- To evaluate the microbiological stability of Usheeradi Taila during the specified storage period.
- To assess the presence or absence of microbial contamination at predetermined time intervals.
- To study the effect of varying temperature and humidity conditions on the stability of the finished formulation.
- To ensure adherence to appropriate **standardization and quality-control parameters** during the preparation and storage of Usheeradi Taila.

MATERIALS AND METHODS:

Collected sample of Usheeradi Taila (sterile packaging and stored at room temperature) studied to check microbial contamination at regular intervals for a period of eight months (till drug was used). Microbiological study has been carried out in Microbiology Laboratory, I.T.R.A., Jamnagar. Studies have been carried out to rule out that presence of any bacteria or fungi in the prepared drug as a final finished product. Initially microbiological study was done on 35^{th} day of preparation before starting patient enrollment and done till the completion of the study regularly with random intervals during different seasons.

DRUG MATERIAL:

One part of course powder of the mentioned drug will be will be boiled appropriate quantity of water and Kashaya will be prepared. To make Kalka, fine powder of the same drugs will be mixed adequate quantity of water. Murchita Tila Taila will be taken in a steel vessel and preheated on mild fire thereafter, once the Taila become warm, Heating is stopped and the prepared Kashaya and Kalka will be added, mixed thoroughly and heated over Mandagni till it attains the Mandhyam Paka Lakshana. Then Taila will be filtered through a clean cloth while it is still warm in to vessel after cooling the Taila will be packed in an airtight glass container to protect the formulation from light and moisture

Table 1: Ingredients Usheeradi Taila [2]

Sr.no.	Name of the drug	Botanical/Latin name	Part used	Parts
1	Usheera	Vetiveria zizanoides Linn.	Root	1
2	Lodhra	Symplocos racemose Roxb.	Bark	1
3	Haritaki	Terminlia chebula Retz	Fruit	1
4	Bibhitaki	Terminlia bellirica Roxb	Fruit	1
5	Amalaki	Phyllanthus emblica L.	Fruit	1
6	Priyangu	Callicarpa macrophylla Vahl	Flower	1
7	Tila Taila	Sesamum indicum	Seed	4

Date of Drug Preparation:14.05.2025 Packing and Storage**:**

Taila was packed in plastic air tight bottles in aseptic area. It was stored in dry place away from direct

sunlight and moisture.

Microbial profile:

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

1. SMEAR EXAMINATION-

A) 10% K.O.H. Preparation

B) Gram's stain

2. CULTURE STUDY-

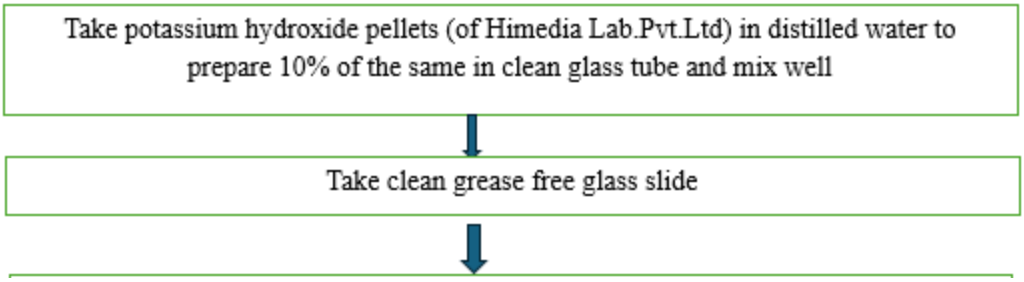
A) Fungal culture

B) Aerobic culture

The details of the procedures followed are given below:

A. SMEAR EXAMINATION: -

1. <u>PROCEDURE FOR 10% KOH PREPARATION:</u>



Put a drop of *Usheeradi Taila* 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 pressure (40x)lens

↓

Report as per findings

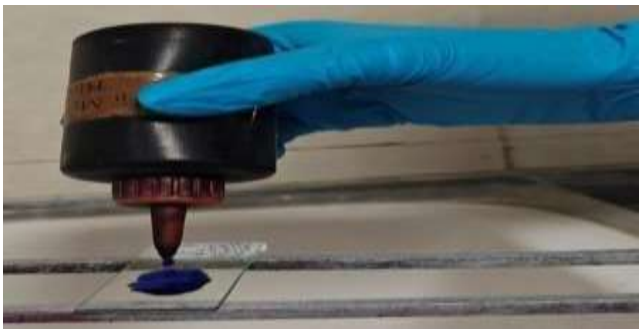


Figure 1. Smear staining Procedure

2. GRAM'S STAIN TEST:

Procedure for Gram Stain

Take clean grease free glass slide to prepare dry equal thick preparation (i.e. smear of *Usheeradi Taila*

Dye (i.e. gram's crystal violet) or as per kit procedure Cover smear with Saffranin solution and allow to remain for mentioned time as per kit procedure

permeable to stain, make material stick to the surface of slide and 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

↓

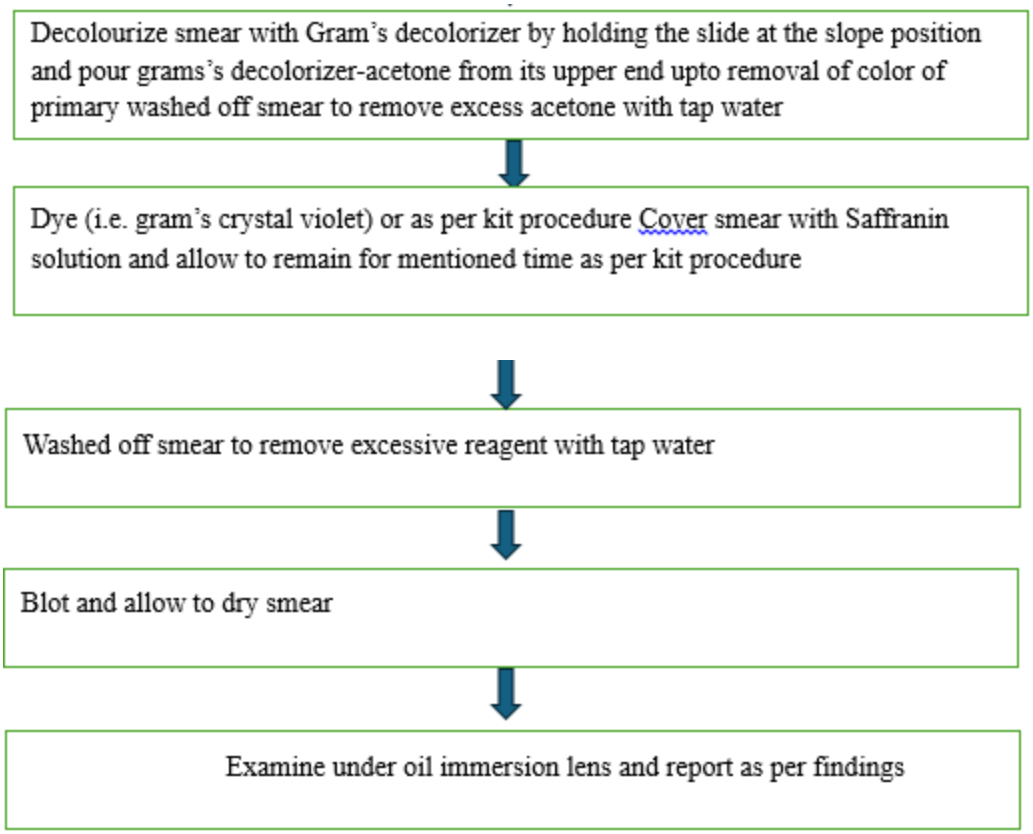




figure no 2 Stained Smear procedure

B. CULTURE STUDY: -

1. 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:

Sabouraud Dextrose Agar Base (SDA) selected as a solid media for inoculation purpose

↓

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

↓

Dry selective solid media in hot air oven before specimen inoculation, allowed to cool dried medium before specimen inoculation

↓

Inoculate *Usheeradi Taila* By sterile cotton swab or by Nichrome wire loop(First sterile loop in Bunsen burner oxidase flame blue flame and allow it to cool than loop was charged with *Usheeradi Taila* and cultured .one loop full of *Usheeradi Taila* was transferred on to the surface of well dried culture media

↓

After inoculation/streaking process, incubate inoculated medium kept in inverted position at 37^0C for 05 to07 to 21 days in Incubator (incubation days are as per growth requirement under aerobic atmosphere)

↓

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



Figure 3 Fungal culture media preparation with Sabouraud Dextrose Agar Base (SDA)

1. AEROBIC 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: 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.

Procedure For Aerobic Culture: -

Mac Conkey Agar (MA) was selected as 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 *Usheeradi Taila* by four flame method (the loop should be flamed and cooled between the different sets of streaks i.e. four time)

↓

On the 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 was charged with *Usheeradi Taila* to be culture

↓

After streaking process incubate inoculated medium in inverted position at 37^0C for 18 to 24 hrs in incubator under aerobic or 10% CO2 atmosphere

↓

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

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

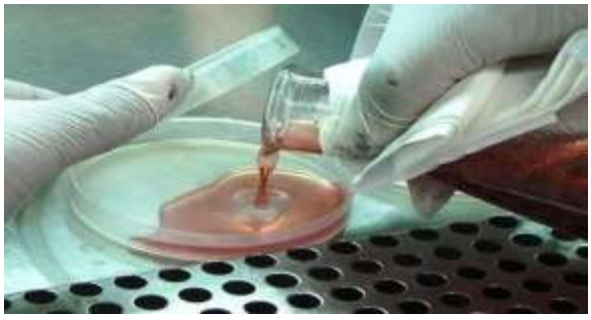


Figure 3 Aerobic culture media preparation with MacConkey Agar (MA)



Figure no 4 Mac Conkey Agar (MA)

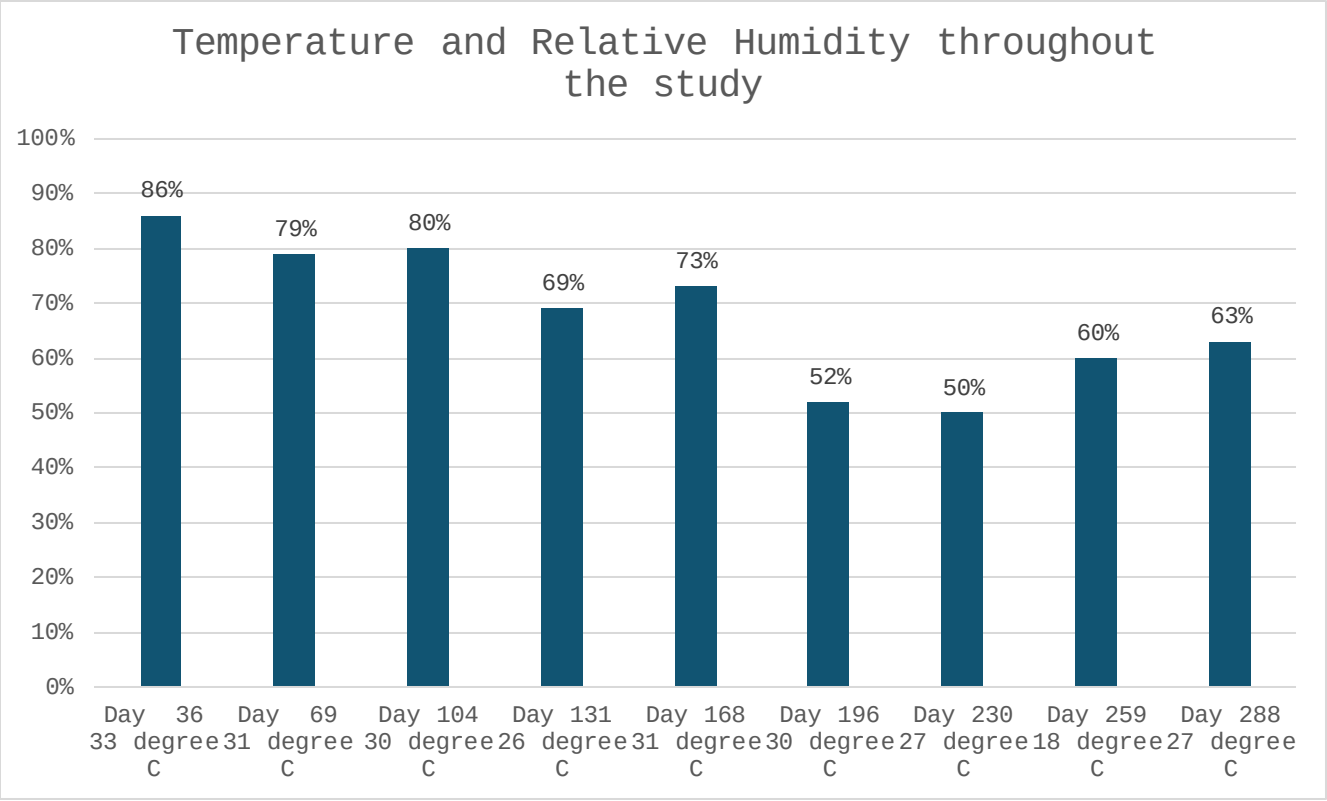
Every time sample (in which drug preserved) were subjected to the microbiological study from the date of the preparation to the date of last microbiological study.

RESULTS:

Shown in table no 2. of Usheeradi Taila. Date of Drug Preparation: 31-05-2025

Sr. No.	Date & Days of investigations After preparation of the sample	Temperature (° C)	Humidity (%)	Observations of sample			
				Gram's Stain	Aerobic culture	Wetmount/ 10% KOH Preparation	Fungal culture
1.	19/06/2025 36th Day	33° C	86%	Microorganism not seen	No organisms isolated	No filaments not seen.	No Fungal Pathogen Isolated
2.	22/07/2025 69 th Day	31° C	79%	Microrganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
3.	26/08/2025 104th Day	30° C	80%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
4.	22/09/2025 131 thDay	26° C	69%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
5.	29/10/2025 168 th Day	31° C	73%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
6.	26/11/2025 196 th Day	30° C	52%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated

7.	**30/12/2025** **230 th Day**	**27° C**	**50%**	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
8.	**28/01/2026** **259 th Day**	**18° C**	**60%**	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
9.	**26/02/2026** **288 th Day**	**27° C**	**63%**	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated



Temperature and relative humidity throughout the study

Ayurveda has a systemic protocol in the management of Prathama Patalagata Timira. Nasya is the Vishesh chikitsa for Timira. Usheeradi Taila is a formulation specially mentioned for Timira .A research work was planned on Usheeradi Taila Nasya at ITRA to break the pathology of Prathama Patalagata Timira (Myopia) as it is one of the challenging issues faced by modern ophthalmologists due to un satisfactory management and high progression rate .Microbial study investigated contamination and stability of Usheeradi Taila across varying temperatures and humidity levels. Usheeradi Taila prepared and stored in temperatures ideal for bacterial growth at room temperature, minimum temperature 18˚C to maximum temperature 35˚C, astoundingly remains microbe- free. Situated in Jamnagar's coastal region, known for its high relative humidity throughout the year, defied expectations. Despite Relative Humidity levels ranging from lowest range 50% on 26th November, 2025 to highest range 86% on 19st June , 2025 as shown in Table 2, no bacterial or fungal growth was observed till study was completed.

Thus, a baseline microbial profile was studied at regular interval of 30 days average upto consumption of

Usheeradi Taila. This indicates that no microbial growth was observed throughout the study, affirming the efficacy of the manufacturing and storage procedures.

CONCLUSION: -

The microbiological assessment of Usheeradi Taila concludes its robust shelf life, remaining free from microbial growth, both bacterial and fungal, for a significant nine -month duration until its scheduled utilization by February 28th 2026, affirming its consistent quality and adherence to standard storage practices.

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