



# A BASELINE MICROBIAL STABILITY STUDY OF *KARPASASTHYADI TAILA* USED IN TREATMENT OF *VATAJA PRATISHYAYA* (ALLERGIC RHINITIS).

Harikrishnan N Menon<sup>1</sup>, Dr. M. S. Cholera<sup>2</sup>, Dr. R. S. Unadkat<sup>3</sup>

1. PhD Scholar, Department of Shalakya, ITRA, Jamnagar.
2. Head Microbiology Laboratory, ITRA, Jamnagar.
3. Reader., Department of Shalakya, ITRA, Jamnagar.

**Corresponding Author:**

Harikrishnan N Menon., PhD scholar, Dept. of Shalakya Tantra,  
ITRA, Jamnagar, Gujarat, India

## ABSTARCT:

Allergic Rhinitis is a disorder of nose induced by an IgE mediated inflammation after Allergen exposure leading to rhinorrhea, nasal pruritus, bilateral nasal stuffiness associated with cough, chest tightness. Worldwide it effects between 10-25% of the population. In India it constitutes more than 50% of all allergies, 20 -30% people suffer from it, and are prone to bronchial asthma in later stage. In Ayurveda it can be correlated with *Vataja Pratishyaya*, for which *Nasya* is considered as one of the main treatment modality, thus we are using *Karpasasthyadi Taila* for *Nasya*. In present study, to check the stability and microbial contamination of the finished product (*Karpasasthyadi Taila*) at different time interval at different climatic conditions, temperature and humidity set ups was carried out.

**Materials and Methods:** In present study, stability with respect to its Microbial profile of *Karpasasthyadi Taila* was carried out. It was stored in plastic container during different climacteric conditions were studied at regular intervals for a period of 10 months to analysis presence bacteria or microbes by Smear and culture study respectively. At the end of study *Karpasasthyadi Taila* had no presence of microbes or bacteria throughout the study 10 months from the day sample preparation even in different climate and temperature.

**Result:** Hence in present study the stability test of *Karpasasthyadi Taila* with respect to microbiological findings was negative at room temperature, warm and cold, dry and humid conditions.

**KEY WORDS:** Microbial profile, Stability, *Karpasasthyadi Taila*

## INTRODUCTION:

*Pratishyaya* (rhinitis) is one among the common diseases affecting respiratory system. In Ayurveda, *Acharya Sushruta* has described 31 *Nasa Roga* (diseases of nose) among them a separate chapter has been devoted to *Pratishyaya* in *Uttartantra*.<sup>[1]</sup> It shows the importance of the same; *Vataja Pratishyaya* (rhinitis due to *Vata*) is one among the five types of *Pratishyaya*.<sup>[2]</sup>

It is a common *Nasagatharoga* characterised by *Bhrisha Kshavathu* (excessive sneezing tendency), *Ahcha Kapha Sruti* (discharge of clear mucous), *Ghranoparodha* (blockage of nose), *Nistoda* in *Shankhapradesha* (pricking pain in temple), *Shirovyatha* (Distress in head or pain) and *Shosha* in *Gala* (Dryness of throat), *Keetika Eva Sarpanti* (feeling as if insects are crawling/pruritus), *Swaropaghata* (Hoarseness of voice).<sup>[3]</sup> Based on above symptoms it can be compared with Allergic Rhinitis (ICD-J30.2, J30.89).<sup>[4]</sup>

*Nasya* (errhine therapy) is a line of treatment for most of the *Urdhwa Jatru Vikaras* (treatment of disease above clavicle) *Acharya Vagbhata* has mentioned that the *Nasya* which is administered for the treatment of *Ardita* (facial paralysis) can be taken for treating *Vataja Pratishyaya*,<sup>[5]</sup> so by following this advice we have considered here to take *Karpasasthyadi Taila* for *Nasya*, which consists of drugs which are having *Ushna Virya* (hot potency) and *Vatahara* (relieves *Vata*) action. Considering the pharmacological properties of all the 15 known drugs it may be said the combination may help in clearing the mucus by thinning it and removal of airborne allergens and inflammation mediators such as histamine, it may also improve the ciliary beat function which may eventually lead to the improvement in muco-ciliary function.<sup>[6]</sup>

The use of herbal medicine has increased remarkably in line with the global trend of people returning to natural therapies. The growing use of botanicals (drug and other products derived from plants) by public if forcing moves to assess the health claims of these agents and to develop standard of quality and manufacture. Microbiological analysis is performed for the estimation of the no. of viable aerobic micro-organism presence and for detecting the presence of designated microbial species in pharmaceutical substance.

**AIM:** To study the stability and to check microbial contamination in the finished product at different time interval with at different climatic conditions, temperature and humidity set ups.

## Materials and Methods:

Collected sample of prepared *Karpasasthyadi Taila* (stored at room temperature) and finished product studied to check microbial contamination at regular intervals for a period of 10months (upto drug used). Microbiological study has been carried out in Microbiology Laboratory, I.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 13<sup>th</sup> day of preparation before starting patient enrollment. Then samples from same container were subjected to the microbiological study regularly with random intervals during different seasons.

**Drug material:**

The raw drug materials were collected from the pharmacy department, I.T.R.A., Jamnagar.

**Ingredients of *Karpasasthyadi Taila* - (Table 1).**

Sl.No:	Sanskrit name	Latin name	Part used	Ratio
<b><i>Drava Dravyas</i></b>				
1.	<i>Karpasasthi</i>	Gossypium herbaceum	Seed	1 part
2.	<i>Bala</i>	Sida cordifolia	Root	1 part
3.	<i>Masha</i>	Phaseolus mungo	Seed	1 part
4.	<i>Kulattha</i>	Dolichos biflorus	Seed	1 part
5.	<i>Aja Ksheera</i>	Capra aegagrus-hircus	Milk	1 part
<b><i>Sneha Dravya</i></b>				
6.	<i>Tila Taila</i>	Sesamum indicum	Seed	1 part
<b><i>Kalka Dravya</i></b>				
7.	<i>Devadaru</i>	Cedrus deodara	Bark, Heart wood	1/8part
8.	<i>Rasna</i>	Pluchea lanceolate	Leaves	1/8 part
9.	<i>Nagara</i>	Zingiber officinale	Rhizome	1/8 part
10.	<i>Kushta</i>	Saussurea lappa	Root	1/8 part
11.	<i>Sarshapa</i>	Brassica campestris	Seed	1/8 part
12.	<i>Shatapushpa</i>	Anethum sowa	Fruit	1/8 part
13.	<i>Chavya</i>	Piper retrofractum	Root, Fruit	1/8 part
14.	<i>Shigru</i>	Moringa oleifera	Root bark	1/8 part
15.	<i>Punarnava</i>	Boerhavia diffusa	Root, <i>Panchanga</i>	1/8 part

**Date of Drug Preparation: 26<sup>th</sup> August, 2023**

**Storage:**

Finished product of *Karpasasthyadi Taila* was stored in air-tight food grade, plastic container, 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.<sup>1</sup>

**1. SMEAR EXAMINATION-**

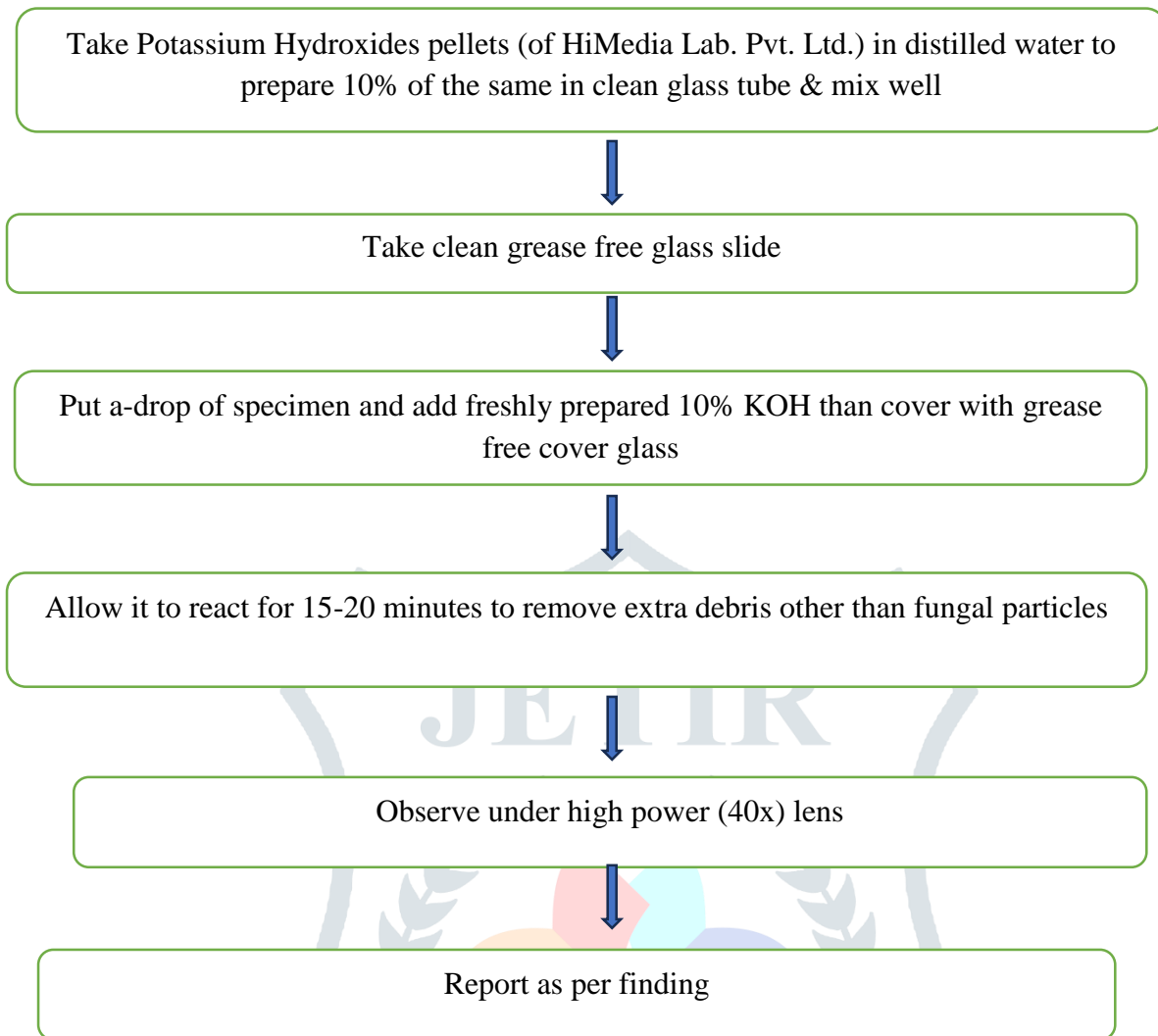
- A) 10% K.O.H. Preparation
- B) Gram's stain

**2. CULTURE STUDY-**

- A) Fungal culture
- B) Aerobic culture

The details of the procedures followed are given below:

**A. SMEAR EXAMINATION: -**

**1. PROCEDURE FOR 10% KOH PREPARATION:**

## 2. GRAM'S STAIN TEST:

### 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 to remain 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 upto removal of colour of primary dye (i.e. Gram's Crystal violet) or as per kit procedure

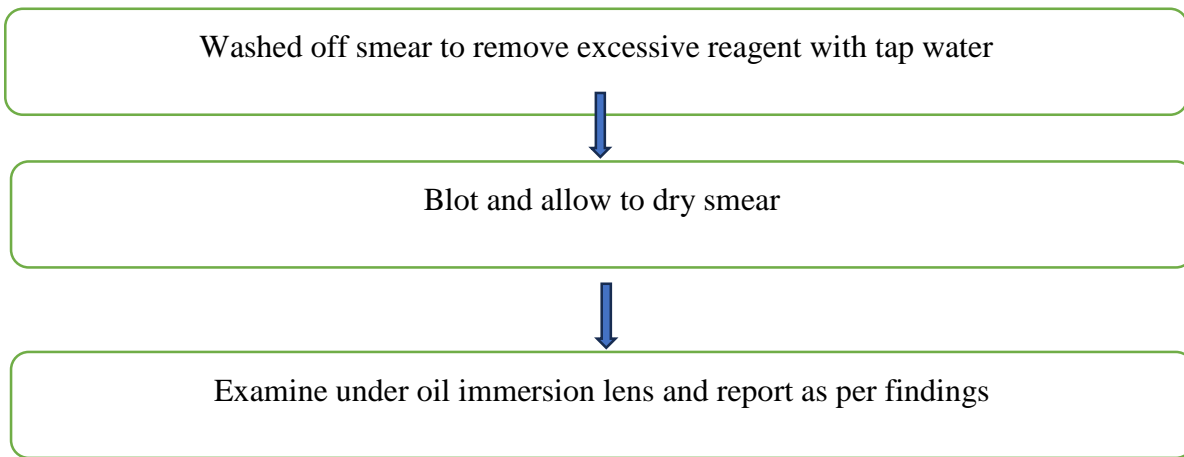


Washed off smear to remove excess acetone with tap water



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





## 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 °C

Use of media: For selective cultivation of pathogenic fungi.

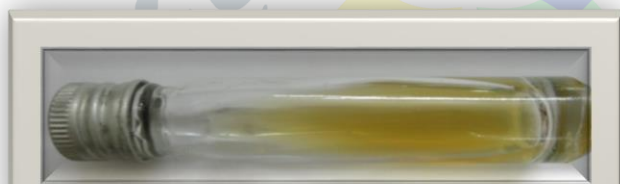
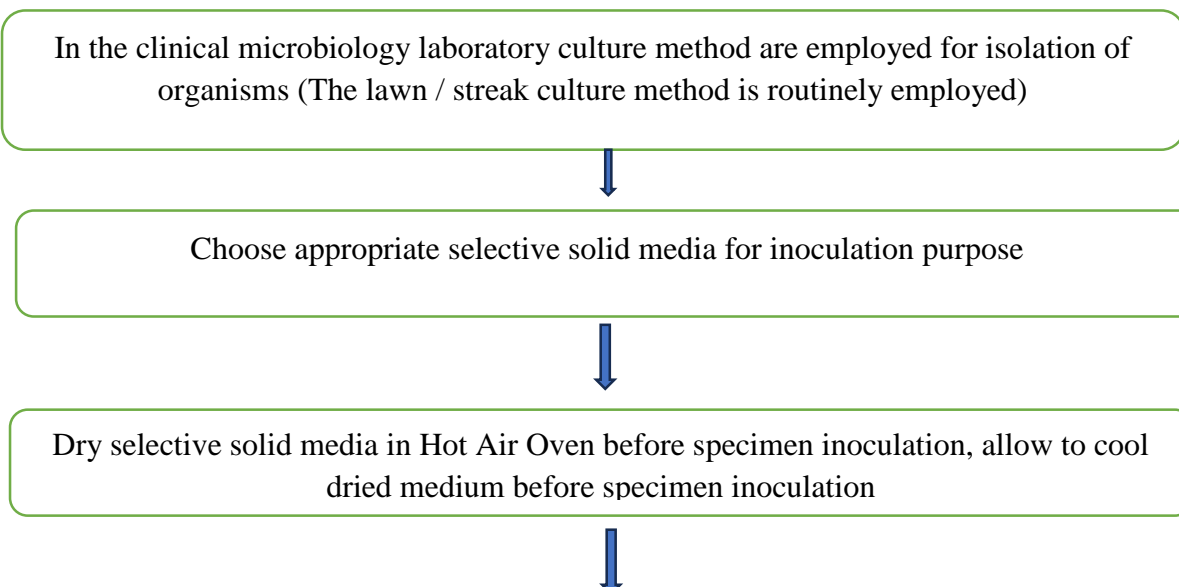


Figure3. Sabouraud Dextrose Agar Base (SDA) bottle

### Procedure For Fungal Culture:



Inoculate selected specimen by Sterile cotton swab or by Nichrome wire (24 5.W.G. size) loop [First sterile loop in Bunsen burner oxidase flameblue 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 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 arial growth and confirm growth by performing different related biochemical reactions and different related staining procedures. After that report isolates.



**Figure 4: Procedure for Fungal culture**

## 2. 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 °C

Use of media: For selective cultivation of pathogenic bacteria.



**Figure 5:MacConkey 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)



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



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



After streaking process Incubate inoculated medium in inverted position at 37°C for 18-24 hours in Incubator under aerobic or 10% CO<sub>2</sub> atmosphere.



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



**Figure 6: 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.

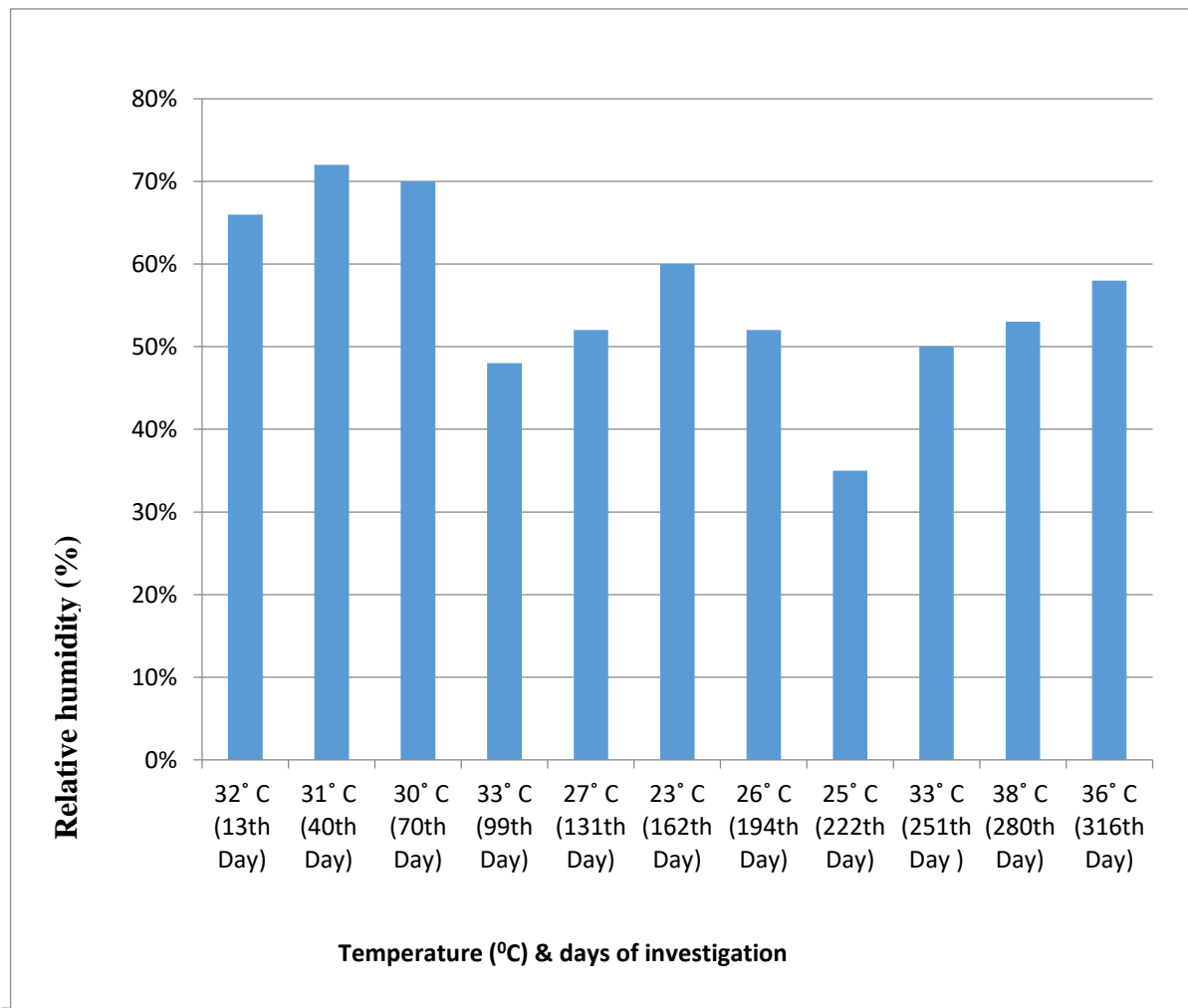
**RESULTS: Shown in table no 1. of Karpasasthyadi Taila.**

- **Date of Drug Preparation: 26/07/2023**

**Table 1: Showing observations of sample preserved at room temperature after preparation of *Taila*.**

Sr. No.	Date & Days of investigations After preparation of the sample	Temperature (° C)	Humidity (%)	Observations of sample			
				Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
1.	08/08/23 13 <sup>th</sup> Day	32° C	66%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
2.	04/09/23 40 <sup>th</sup> Day	31° C	72%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
3.	04/10/23 70 <sup>th</sup> Day	30° C	70%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
4.	02/11/2023 99 <sup>th</sup> Day	33° C	48%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
5.	04/12/23 131 <sup>th</sup> Day	27° C	52%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
6.	04/01/24 162 <sup>th</sup> Day	23° C	60%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
7.	01/02/24 194 <sup>th</sup> Day	26° C	52%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated

8.	04/03/24 222 <sup>th</sup> Day	25° C	35%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
9.	02/04/24 251 <sup>th</sup> Day	33° C	50%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
10.	01/05/24 280 <sup>th</sup> Day	38° C	53%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
11.	06/06/24 316 <sup>th</sup> Day	36° C	58%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated



**DISCUSSION:** Ayurveda as an adjuvant therapy is widely used in *Vataja Pratishyaya* (Allergic rhinitis). *Karpasasthayadi Taila* used for *Nasya*, as it does the *Vata hara* action along with removing the *Dooshita Dosha* from the *Jatru urdwa pradesha*. In present study, it has shown a very good and promising result. Hence, the microbial study was carried out to observe the stability study of *Karpasasthayadi Taila* with respect to microbial contamination of sample prepared and preserved in different climatic and temperature conditions. Stability is usually expressed in term of shelf-life, which is the time period from when the product is produced until the time it is intended to be consumed or used. Microorganism needs water, humidity and temperature at suitable environmental conditions to develop in any media, surface or article temperature setups ranged from minimum temperature 25 °C to maximum temperature 41 °C, which proved as standard temperature for various typed of bacteria to overgrow. At the end of study, it was found that sample was not showed presence of any

microbes which was preserved at room temperature at Jamnagar region. High Relative Humidity (RH) allows the growth of the microbes. Here *Karpasasthayadi Taila* which was prepared and stored at the Jamnagar region, proximal to sea coast, where RH remains high throughout a year. As shown in Table 2, highest humidity was 72 % on 4<sup>th</sup> September, 2023 while lowest was 35 % on 4<sup>th</sup> March, 2024. Although RH remained variable throughout the study period, microbial growth was not observed during the study. Thus, a baseline microbial profile was studied at regular interval of 30 days average up to the use of *Karpasasthayadi Taila*. This indicates that manufacturing and storage procedure adopted in this study was up to the mark.

**CONCLUSION:** Hence Microbiological study of the *Karpasasthayadi Taila* showed that the quality of *Taila* is in a standard condition. There were no growth found of microorganisms (bacterial or fungal), till 4<sup>th</sup> June 2024i.e. 10 months (upto consumption of drug) from the date of preparation, shows its good shelf life.

**FINANCIAL SUPPORT:**

The study was supported by the Institute of Teaching and Research (ITRA), Jamnagar, Gujarat, Bharat

**CONFLICTS OF INTEREST:**

There are no conflicts of interest.

**REFERENCE: -**

- 1) Kaviraj Ambikadutta Shastri, editor.Susruta Samhita of Maharsi-usruta,Uttaratantra.Volume 2. Ch.22, Ver. 4.ed. Varanasi: Chaukhambha Sanskrit Sansthan; 2018. P.135.
- 2) Kaviraj Ambikadutta Shastri, editor.Susruta Samhita of Maharsi-Susruta,Uttaratantra. Volume 2. Ch.22, Ver. 5.ed. Varanasi: Chaukhambha Sanskrit Sansthan;2018. p. 135.
- 3) Vagbhata. Astanga Hrudaya, Translated by late Dr. Anna Moreshwara Kunte, Reprint. varanasi: Chaukhambha Krishnadas Academy; Uthara sthana,19/4 p.683.
- 4) <http://www.icd10data.com/search?s=allergic> rhinitis [accessed on 05/06/2024 at 11 pm].
- 5) Vagbhata. Astanga Hrudaya, Translated by late Dr. Anna Moreshwara Kunte , Reprint. varanasi: Chaukhambha Krishnadas Academy; Uthara sthana,20/10 p.692.
- 6) Sreeja V S. Vikram Kumar. Standardization of Nasya dose by Bindu Pramana with Karpasasthyadi Taila. International Ayurvedic Medical Journal. Volume 6. Issue 4. P. 1200-1204. [http://iamj.in/current\\_issue/images/upload/1200\\_1204.pdf](http://iamj.in/current_issue/images/upload/1200_1204.pdf)
- 7) Ananthanarayana and Paniker's, Text book of Microbiology, edition 10<sup>th</sup>, chapter 4- Culture media, page 39-43 and Chapter 5, Culture methods page- 44-47.