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Microbial Evaluation of Apamargakshara Taila for Karnanaada (Tinnitus): A Stability Study

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ABSTRACT:
Tinnitus is one of the common and prevalent ailments which can be correlated symptomatically to *Karnanaada*. Tinnitus is characterized by annoying ear noises which can be soft as a whistle or loud enough to be completely debilitating where the origin of this sound is within the patient[i]. Tinnitus is the most prevalent otological condition, which may have a great impact on the quality of life of sufferers as well as their family members. As the prevalence of tinnitus is high among adult population, it is very much essential to develop proper assessment and management protocols to help the patients with tinnitus. *Apamargakshara Taila* is one of the best choices in the management of *Karnanaada* (Tinnitus). It is very essential to check the stability and to check microbial contamination in the finished product at different time interval- at different climatic conditions, temperature and humidity set up.
Materials and Methods: In present study, stability with respect to its Microbial profile of *Apamargakshara Taila* was carried out. It was stored in plastic bottle during different climatic conditions and were studied at regular intervals for a period of eight months to analysis presence no microbial growth by Smear and culture study respectively. At the end of study *Apamargakshara Taila* had no presence of microbes or bacteria throughout the study eight months from the day sample preparation even in different climate and temperature.
Result: In present study, the stability test of *Apamargakshara Taila* with respect to microbiological findings was negative at room temperature, warm and cold, dry and humid conditions.

KEYWORDS: *Karnanaada,* Microbial profile, Stability, *Apamargakshara Taila*

INTRODUCTION:

Tinnitus is one of the common and prevalent ailments which can be correlated symptomatically to *Karnanaada*. Tinnitus is characterized by annoying ear noises which can be soft as a whistle or loud enough to be completely debilitating where the origin of this sound is within the patient. Tinnitus is the most prevalent otological condition, which may have a great impact on the quality of life of sufferers as well as their family members. As the prevalence of tinnitus is high among adult population, it is very much essential to develop proper assessment and management protocols to help the patients with tinnitus. *Apamargakshara Taila* is one of the best choices in the management of *Karnanaada* (Tinnitus).
Karnapoorana means filling of ear with luke warm *Taila*, *Swarasa* or *Mutra* of animals. *Karnapoorana* comes under *Bahya Snehana* and is a *Vishesha Chikitsa* of *Karnanaada. Apamargakshara Taila Karnapoorana* is ideal in the management of *Karnanaada* (Tinnitus).

Microbiological analysis is performed for the estimation of the number of viable aerobic micro-organism presence and for detecting the presence of designated microbial species in pharmaceutical substance. Shelf- life, a vital metric for product stability, marks the duration between production and consumption. Microbial growth thrives within specific environmental parameters: temperatures ranging from 20°C to 40°C (68°F to 104°F) and relative humidity levels of 60% to 90%. These conditions offer an ideal breeding ground for bacteria and fungi on surfaces and articles. Maintaining control within this range is critical for preserving product quality. Understanding these factors facilitates the implementation of effective storage strategies to curb microbial contamination and prolong shelf-life. Precise temperature and humidity regulation uphold product integrity, ensuring consumer confidence and safety.

AIM: Comprehensively to assess the stability of *Apamargakshara Taila* (finished product) and monitor microbial contamination under varying time intervals, diverse climatic conditions, and fluctuations in temperature and humidity.

OBJECTIVE: -To ensure adherence to rigorous standardization protocols in the production of study drugs, thereby enhancing overall quality control and efficacy.

MATERIALS AND METHODS:

Collected sample of *Apamargakshara 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 6^{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:

The raw drugs *Apamarga* and *Tila Taila* was taken from the pharmacy of ITRA, Jamnagar. *Apamargakshara Taila* was prepared with *Apamarga Kalka*, *Apamarga Kshara Jala* and Tila Taila. *Apamarga Kalka* was prepared using fresh *Apamarga Samoola.*

For the preparation of *Apamarga Kshara*, dry *Apamarga* was burnt in an open pan till it becomes a white powder. All the organic matter was burnt, to get the white colour *Kshara*. *Kshara* is the water-soluble ash left after the burning of organic substances. *Kshara* is mainly the Potassium and Sodium portion of the plant along with the chlorides, sulphates, sulphides, phosphates, magnesium, and so on. It was prepared in a stainless-steel vessel to prevent reaction between the vessel and *Kshara*. *Apamarga Kshara* was strained and four times (v/v) water was added. It was soaked and kept overnight and again strained thrice. After evaporation of the water portion, the white colour powder was collected. *Apamargakshara Jala* was prepared by adding four times (w/v) water into *Kshara*. During the preparation of *Apamargakshara Taila*, four parts of *Tila Taila* was taken in a wide mouthed stainless-steel utensil and heated till fumes emerged from the oil. The *Taila* was warmed, and one part of *Apamarga Kalka* and sixteen parts of *Apamargakshara Jala* were added. It was continuously stirred. Cooking was done on moderate heat. When the entire watery portion had evaporated (*Sneha Siddhi Lakshana*), the oil was strained in a warm condition. It was collected in a glass bottle. The whole procedure was completed within three days.

Table 1: Ingredients *Apamargakshara Taila*- [ii]

No.	*Sanskrit Name*	*ame/ English Name*	*Part Used*	*Part*
1	*Apamarga Kalka*	*Achyranthes aspera Linn.*	*Samoola (fresh)*	*1*
2	*Tila Taila*	*Sesamum indicum Linn.*	*Taila*	*4*
3	*Apamarga Kshara Jala*	*Achyranthes aspera Linn.*	-	*16*

Date of Drug Preparation: 09.05.2024

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.

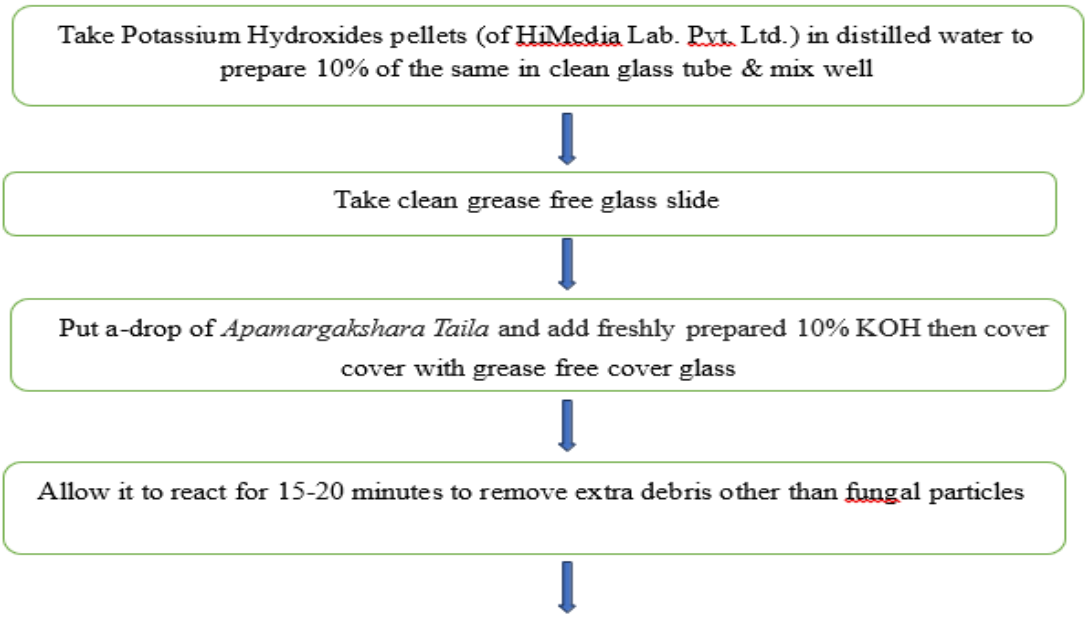
Microbial profile:
Microbial contamination was assessed by two methods to check any mycological findings and bacteriological findings.[iii]

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. PROCEDURE FOR 10% KOH PREPARATION:



Observe under high power (40x) lens

Report as per finding

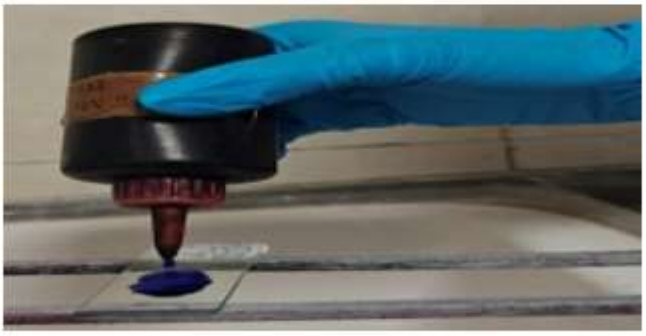


Figure 1. Smear staining Procedure

2. GRAM'S STAIN TEST:

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

Fixed prepared smear (*Apamargakshara Taila*) by passing 3-4times 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

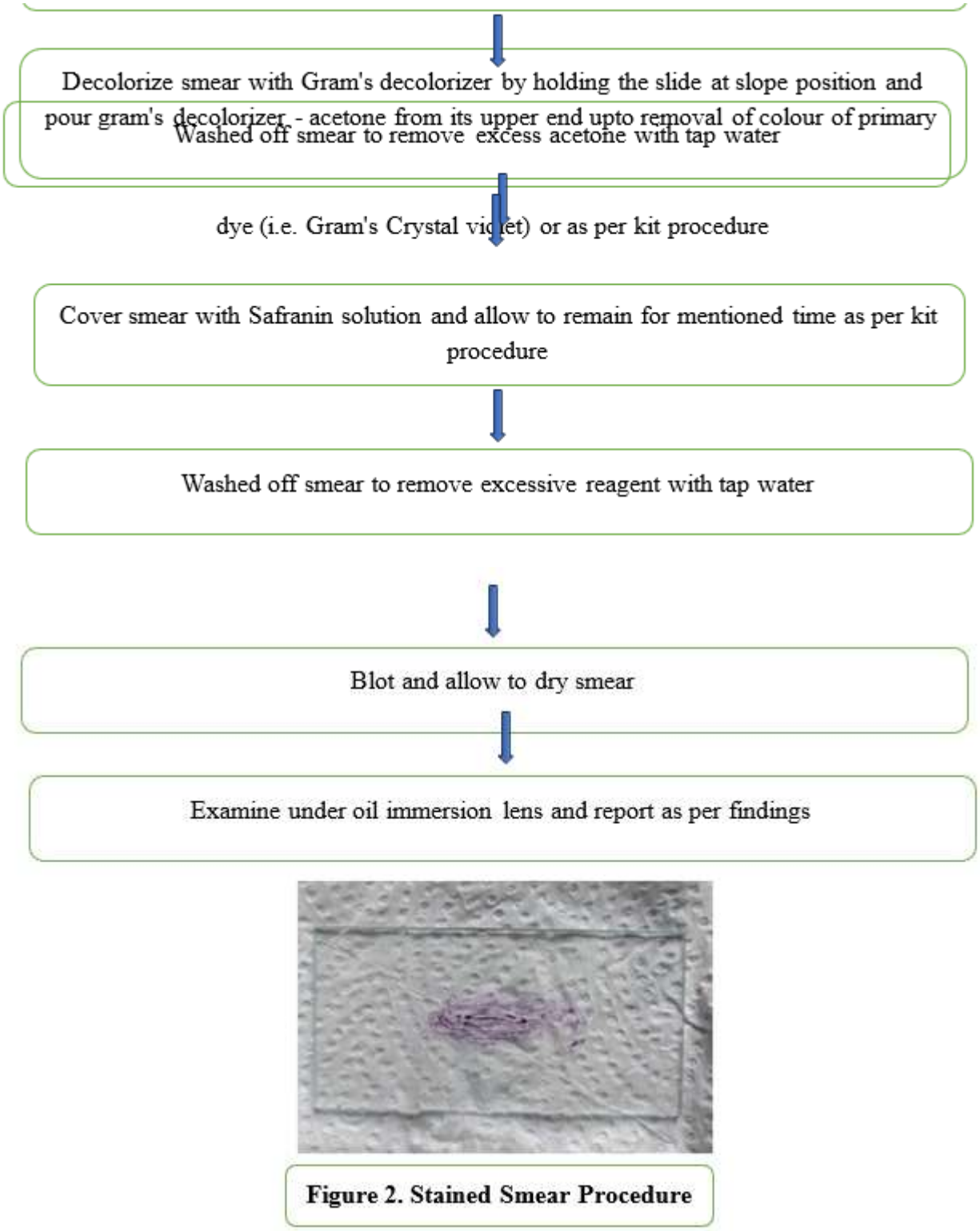


Figure 2. Stained Smear Procedure

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:

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

↓

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

↓

Dry selective solid media in Hot Air Oven before specimen inoculation, allowed to cool dried medium before specimen inoculation

↓

Inoculate *Apamargakshara Taila* 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 was charged with *Apamargakshara Taila* and cultured. One loop full of *Apamargakshara Taila* was transferred onto the surface of well dried culture media]

↓

After inoculation / streaking process, Incubate inoculated medium kept 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 the 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.

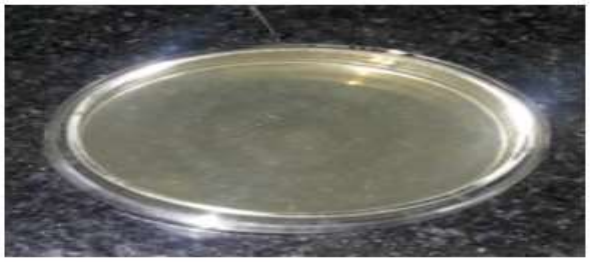


Figure 3 Fungal culture media preparation with Sabouraud Dextrose Agar Base (SDA)

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.

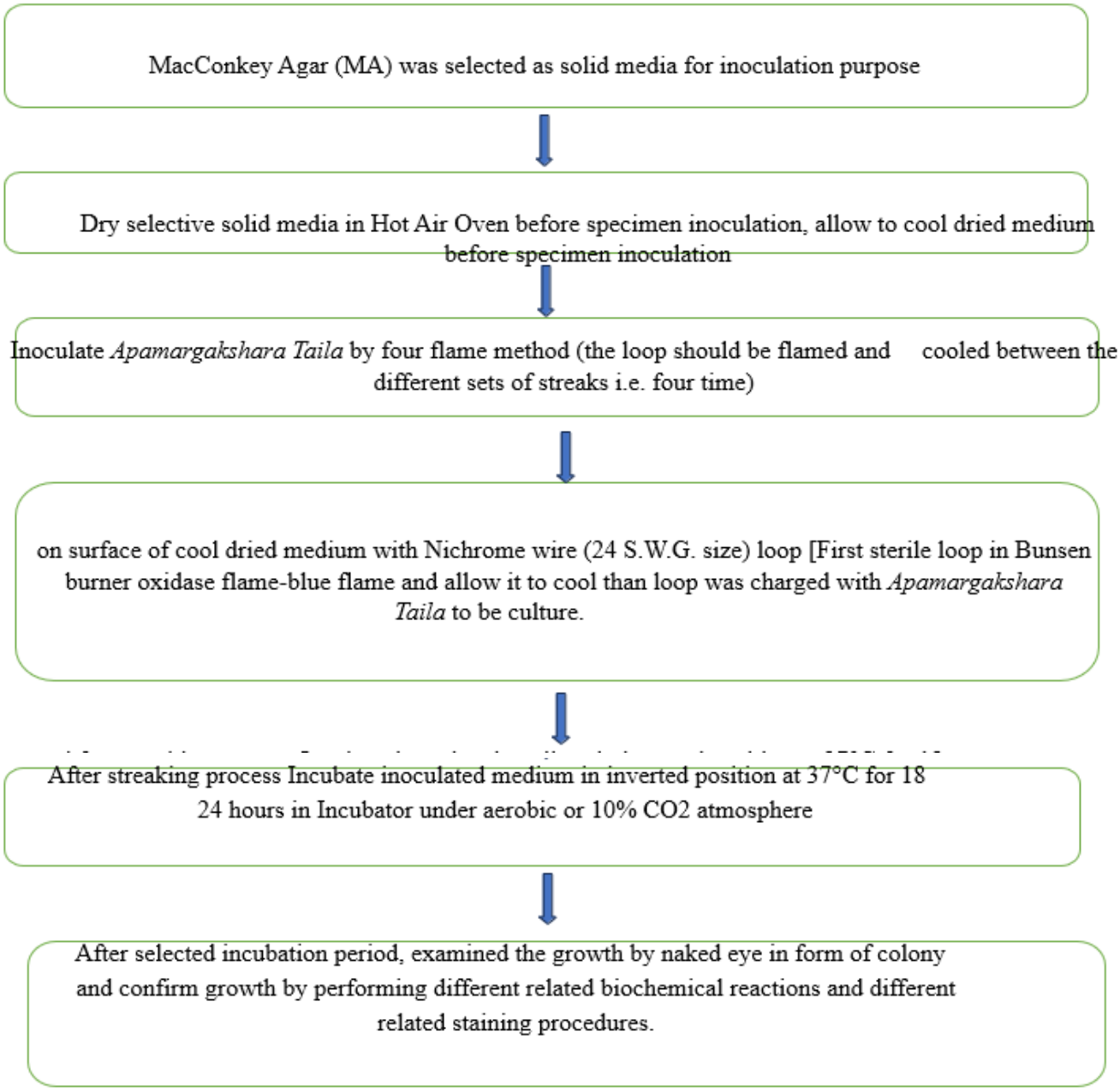
Required time duration: 24 to 48 hours

Required temperature: 37 ºC

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 organisms (The streak culture method is routinely employed)



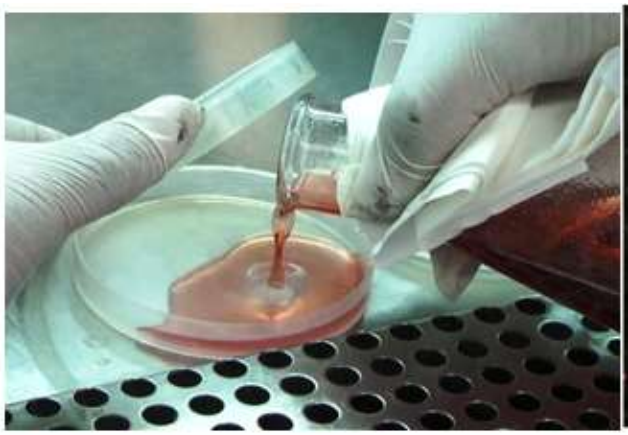


Figure 3 Aerobic culture media preparation with MacConkey Agar (MA)

Figure 4 MacConkey Agar (MA)

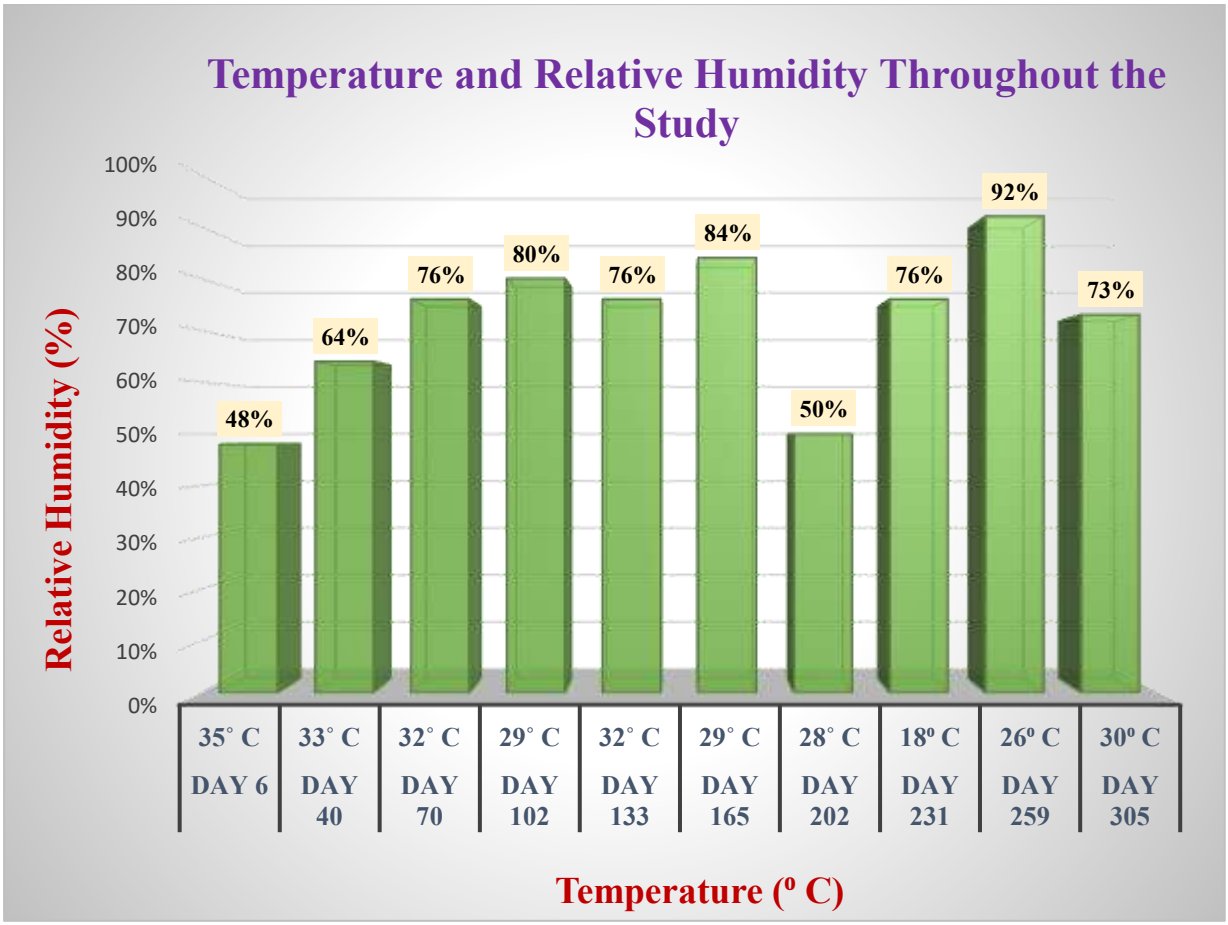
MacConkey Agar (MA) was selected as solid media for inoculation purpose
Dry selective solid media in Hot Air Oven before specimen inoculation, allow to cool dried medium before specimen inoculation
Inoculate *Apamargakshara Taila* by four flame method (the loop should be flamed and cooled between the 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 was charged with *Apamargakshara*
Taila to be culture.
After streaking process Incubate inoculated medium in inverted position at 37°C for 18 24 hours 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.
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 *Apamargakshara Taila*.
Date of Drug Preparation: 09.05.2024

Table 2: Showing observations of *Apamargakshara Taila* preserved at room temperature after the preparation of *Taila*[iv]

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.	**16/05/2024 6th Day**	**35° C**	**48%**	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
2.	**18/06/2024 40th Day**	**33° C**	**64%**	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
3.	**18/07/2024 70th Day**	**32° C**	**76%**	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
4.	**19/08/2024 102nd Day**	**29° C**	**80%**	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
5.	**19/09/2024 133rd Day**	**32° C**	**76%**	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
6.	**21/10/2024 165th Day**	**29° C**	**84%**	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
7.	**27/11/2024 202nd Day**	**28° C**	**50%**	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
8.	**26/12/2024 231st Day**	**18° C**	**76%**	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
9.	**23/01/2025 259th Day**	**26° C**	**92%**	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
10.	**10/03/2025 305th Day**	**30° C**	**33%**	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated



Ayurveda has a systemic protocol in the management of *Karnanaada. Karnapoorana* is the *Vishesha Chikitsa* for *Karnaroga. Apamargakshara Taila* is formulation specially mentioned for *Badhirya* and *Karnanaada.* A research work was planned on *Apamargakshara Taila* ay ITRA to break the pathology of *Karnanaada* (Tinnitus) as it one of the challenges issues faced by modern ENT surgeons due to unsatisfactory management and high recurrence rate.

A microbial study investigated contamination and stability of *Apamargakshara Taila* across varying temperatures and humidity levels. *Apamargakshara 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 33% on 10th March, 2025 to highest range 92% on 23rd January, 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 *Apamargakshara 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 *Apamargakshara Taila* concludes its robust shelf life, remaining free from microbial growth, both bacterial and fungal, for a significant eleven month duration until its scheduled utilization by March 30th 2025, affirming its consistent quality and adherence to standard

storage practices.

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