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Microbial Evaluation and Comparison of Stability of Vasaharitaki Avaleha with Different Diagnostic Modalities Preferred for Vataja Pratishyaya (Allergic Rhinitis): A Stability Study

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ABSTRACT:
Many research works have been carried out on Vataja Pratishyaya (Allergic Rhinitis) in Ayurveda and also in contemporary system of medicine, Vataja Pratishyaya (Allergic Rhinitis) still remains as a hazardous problem as it has more chances of recurrent episodes and serious complications. Vasaharitaki Avaleha is a classical Ayurvedic formulation widely indicated in the management of Kasa (cough), Shwasa (respiratory disorders), and Pratishyaya (rhinitis) due to its Kapha-Vata Shamaka and Rasayana properties. The formulation is believed to facilitate mucolytic and expectorant actions, improve respiratory function, and support respiratory health by promoting balancing of Doshas and enhancing tissue rejuvenation. So, the study has been planned to find out the management from the ancient texts which is effective and cheap. 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 Vasaharitaki Avaleha was carried out. It was stored in plastic bottle during different climatic conditions and were studied at regular intervals for a period of seven months to analysis presence of microbial growth by smear and culture study respectively. At the end of study Vasaharitaki Avaleha had no presence of microbes or bacteria throughout the study seven months from the day sample preparation even in different climate and temperature.
Result: In present study, the stability test of Vasaharitaki Avaleha with respect to microbiological findings was negative at room temperature, warm and cold, dry and humid conditions.

KEY WORDS: Vataja Pratishyaya, Microbial profile, Stability, Vasaharitaki Avaleha

INTRODUCTION:

Allergic Rhinitis is one of the common and prevalent ailments which can be correlated symptomatically to Vataja Pratishyaya. It is characterized by Paroxysmal sneezing, nasal blockage, nasal discharge, headache, heaviness in the head, itching in eyes, throat, palate, etc. Allergies are several conditions caused by hypersensitivity of the immune system to typically harmless substances in the environment.[i] Pollen and certain food, metals are common allergens. Food, insect stings, and medications are common causes of severe reactions. It occurs due to genetic and environmental factors. Vasaharitaki Avaleha exerts its

therapeutic efficacy through Kapha-Vata Shamana, Srotoshodhana, and Rasayana actions, making it a valuable formulation in the management of respiratory disorders. 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 Vasaharitaki Avaleha (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 Vasaharitaki Avaleha (sterile packaging and stored at room temperature) studied to check microbial contamination at regular intervals for a period of seven 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 5th day of preparation before starting patient enrollment and done till the completion of the study and consumption of drug regularly with random intervals during different seasons.

DRUG MATERIAL:

All the herbal dried ingredients of Vasaharitaki Avaleha were made into powder form separately. Vasa Panchanga Yavakuta was washed and dried properly, then Kwatha was prepared by adding 8 times the amount of water as that of Vasa Yavakuta and it was heated to reduce to one fourth. Sita was added to kwatha and boiled upto two string consistency by gradual heating. Then Haritaki Churna and Pippali churna were added and stirred thoroughly during the process. The above powdered Prakshepa dravyas (Twak, Ela, Tamalpatra, Nagakesara) were added and mixed thoroughly to prepare a homogenous mass, allowed to cool at room temperature. After that, Madhu was added and mixed thoroughly. The Vasaharitaki Avaleha was then kept in air tight plastic container.

Ingredients of Vasaharitaki Avaleha- (Bhaishajyaratnavali Chikitsa Prakarana13/107-109). [ii]

Sr. No.	NAME OF DRUG	LATIN/ENGLISH NAME	PART USED	RATIO
1.	Vasa	Adhatoda vasica Nees.	Whole Plant	100 Parts
2.	Haritaki	Terminalia chebula Retz.	Fruit	64 Parts
3.	Pippali	Piper longum Linn.	Fruit	2 Parts
4.	Twaka	Cinnamomum verum Presl.	Stem Bark	1 Part
5.	Ela	Elettaria cardamomum Linn.	Fruit	1 Part
6.	Nagakeshara	Mesua ferrea Linn**.**	Stamen	1 Part
7.	Tamalpatra	Cinnamommum tamala Nees. & Eberm.	Leaves	1 Part
8.	Sita	Saccarum officinarum Linn.	-	100 Parts
9.	Madhu	Myrrhis odorata Linn.		16 Parts

Date of Drug Preparation: 23.05.2025
Packing and Storage: Avaleha was packed in plastic air tight bottles in an aseptic area. It was stored in refrigerator away from direct sunlight and moisture.
Microbial profile: Microbial contamination was assessed by two methods to check any mycological findings and bacteriological findings.[iii]

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:

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 droplet of *Vasaharitaki Avaleha* 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 finding

Figure 1: Smear staining Procedure

2. <u>GRAM'S STAIN TEST:</u>

Procedure for Gram's Stain

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

↓

Fixed prepared smear (*Vasaharitaki Avaleha)* by passing 3-4 times over the flame of Busen 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 the 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 primary Dye (i.e..Gram's Crystal violet) or as per kit procedure

Washed off smear to remove excessive 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

Blot and allow to dry smear

Examine under oil immersion lens and report as per findings

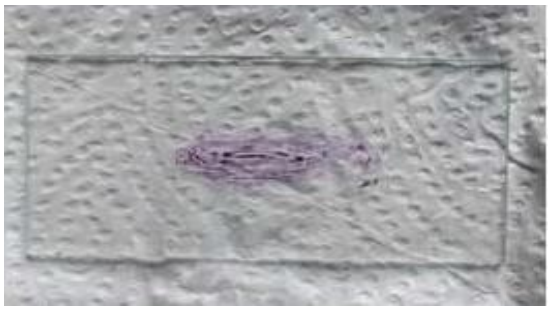


Figure 2. Stained Smear Procedure

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:

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 *Vasaharitaki Avaleha* 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 *Vasaharitaki Avaleha* and cultured. One loop full of *Vasaharitaki Avaleha* 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) underaerobic atmosphere

↓

After selected incubation period, examined the growth by naked eye in form of cokny 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)

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 *Vasaharitaki Avaleha* by four flame method (the loop should be flamed and different sets of streaks ie four time) 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 *Vasaharitaki Avaleha* 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.

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

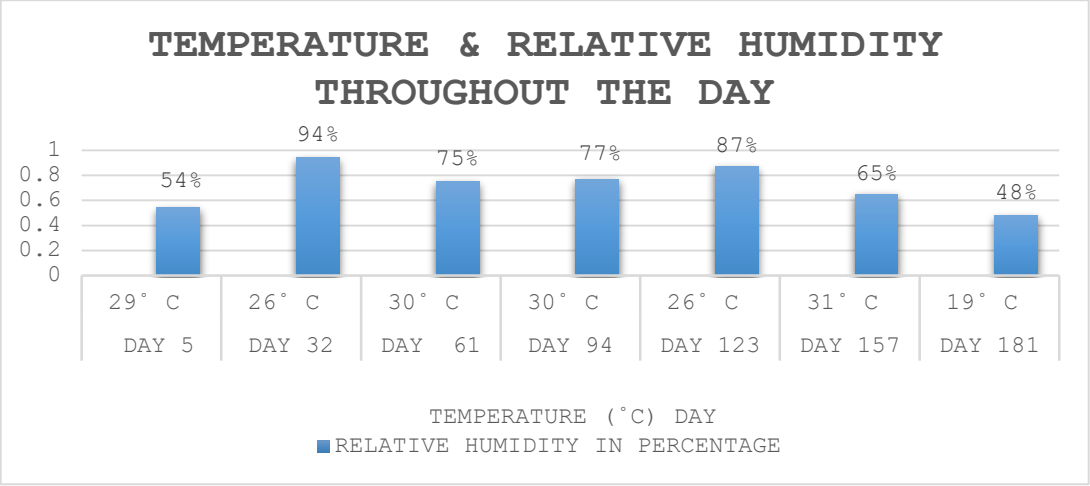
Figure 4 MacConkey Agar (MA)

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 Vasaharitaki Avaleha.
Date of Drug Preparation: 23.05.2025

Table 2: Showing observations of Vasaharitaki Avaleha preserved at room temperature after the preparation of Avaleha[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.	28/05/2025 5th Day	29˚ C	54%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
2.	30/06/2025 32nd Day	26˚ C	94%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
3.	30/07/2025 61th Day	30˚ C	75%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
4.	01/09/2025 94th Day	30˚ C	77%	iMicroorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
5.	30/09/2025 123th Day	26˚ C	87%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
6.	03/11/2025 157th Day	31˚ C	65%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
7.	27/11/2025 181th Day	19˚ C	48%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated



Ayurveda has a systemic protocol in the management of Vataja Pratishyaya (Allergic Rhinitis). Morbidity from the condition can significantly impair patient's quality of life and productivity. Conventional Allopathic therapies, particularly when administered in higher doses or over prolonged periods, may be associated with adverse effects such as septal perforation, rhinitis medicamentosa, drowsiness, and bronchial asthma. Vasaharitaki Avaleha has Rasayana and Rogahara property along with anti-inflammatory and bronchodilator effect. It may give an immune modulator effect which will be effective in the management of Vataja Pratishyaya (Allergic Rhinitis).

A microbial study investigated contamination and stability of Vasaharitaki Avaleha across varying temperatures and humidity levels. Vasaharitaki Avaleha prepared and stored in temperatures ideal for bacterial growth at room temperature, minimum temperature 19˚C to maximum temperature 31˚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 48% to highest range 94% 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 time interval upto consumption of Vasaharitaki Avaleha. 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 Vasaharitaki Avaleha concludes its robust shelf life, remaining free from microbial growth, both bacterial and fungal, for a significant seven-month duration until its scheduled utilization by November 30th, 2025, affirming its consistent quality and adherence to standard storage practices.

DISCUSSION: -

In Year 2021, a shelf-life evaluation of Vasaharitaki Avaleha and Vasaharitaki Granules: An Ayurvedic Polyherbal formulation with its modified dosage form cocluded a stability period of Vasaharitaki Avaleha of 3.3 years by means of an accelerated time stability study,[v] which was found comparable with the present study using different diagnostic modes of stability i.e. bacterial and fungal culture modes as shown in the results and observations.

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