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ORIGINAL ARTICLE

Stability Study *Bramhyadi* Tablet' used in the Management of Pre-Operative Anxiety: with Respect to Baseline Microbial Diagnostic Modalities

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ABSTRACT

The research employs various diagnostic modalities which include microbial enumeration, identification of microbial species, and assessment of antimicrobial activity to establish a baseline microbial profile of the *Bramhyadi* tablet. The "Stability Study of *Bramhyadi* Tablet" focuses on evaluating the stability study in regard of microbial quality of *Bramhyadi* tablets. This study aims to assess the changes in the tablet's physical, and microbiological properties over time (14 months) to determine its shelf life and microbial stability throughout the study period.

The study investigates the effects of different atmosphere and packaging materials on the tablet's stability and microbial contamination by smear and culture examination. The absence of microbial growth in *Bramhyadi* tablet throughout the study provide valuable insights into the shelf life and microbial stability of drug. So, drug is stable at different atmosphere (highest temperature 107° F & 80° F minimum temperature and humidity (Maximum 85% & minimum 13%) and can be stored in plastic zipped bag. The findings help to determine the appropriate storage conditions, expiration date, and recommended microbial quality standards for the product.

KEYWORDS

• Ayurveda • *Bramhyadi* Tablet • Stability Study • Microbial Contamination

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INTRODUCTION

A drug stability study is an important aspect of pharmaceutical development and manufacturing. It involves assessing the physical, and microbiological properties of a drug product over time to determine its shelf life and storage conditions. The stability study provides crucial information on how the drug degrades, whether it remains safe and effective throughout its intended shelf life, and the recommended storage conditions to maintain its quality.

Stability of a pharmaceutical product is a prime essential need to be fulfilled prior to starting clinical study. It is the capability of a particular formulation in a specific container or closure system, to remain within its physical, microbiological, therapeutic, and toxicological specifications at a defined storage condition.

Drug stability study can establish various criteria i.e., Shelf-life determination, Formulation optimization, Regulatory compliance, Quality control, Batch-to-batch consistency.

Ayurveda drug formulary had already given criteria regarding drugs shelf life and its storage in respected chapter. But new formulary (formed based on experience or polyherbal drug) which is not directly described under the classical chapter needs validation regarding its stability for its further usage. This study includes the stability of the "*Bramhyadi* tablet" which is formed by the hydro-alcoholic extract of four Ayurveda drugs i.e., *Bramhi (Bacopa monnieri Linn.), Jatamansi (Nardostachys jatamansi Dc.), Parsik Yavani (Hyoscyamus niger Linn.), Ashwagandha (Withania somnifera Dunal)*.

In such cases, baseline microbial study can be helpful to identify and detect the presence of harmful microorganisms such as bacteria, fungi, yeast, and molds in Ayurveda drugs. This is crucial to prevent the use of contaminated products that may pose health risks to consumers. This study is also helpful to evaluate the effectiveness of manufacturing processes, storage conditions, and packaging in preventing microbial growth. By ensuring that Ayurveda drugs are free from harmful microorganisms, microbial studies contribute to the safety and efficacy of the products. Also provides data and evidence to demonstrate compliance with regulations of drug productions. It is useful supportive data which ensure that Ayurveda drugs meet the necessary standards for microbial purity, preventing potential regulatory issues and ensuring consumer safety.

The study emphasizes the importance of baseline microbial diagnostic modalities in assessing the microbial quality of Ayurvedic formulations and highlights the significance of stability studies in ensuring the safety and efficacy of traditional herbal preparations used in clinical settings. They provide scientific evidence to support regulatory approval, help optimize formulations, and ensure that drugs maintain their quality throughout their intended shelf life. In Nutshell, Stability studies for ayurveda drugs are essential for determining shelf life, ensuring quality, optimizing formulations, complying with regulatory requirements, and ensuring patients safety. They provide scientific evidence to support the efficacy, safety, and stability of ayurveda drugs throughout their intended shelf life.

Aim: To check the stability of *Bramhyadi* tablet along with microbial contamination in tablet in different type of atmosphere (Various Temperature, Humidity)

MATERIALS AND METHODS

Culture report to rule out microbial contamination was carried out every month in the microbiology lab, institute of teaching and research in Ayurveda, Jamnagar.

Raw drug procurement: Raw drug (Dry hydro-alcoholic extract of four drug-Bramhi: *Bacopa monneri Linn., Jatamansi: Nardostachys jatamansi Dc., Ashwagandha: Withania somnifera Linn, Parsik Yavani: Hyoscyamus niger Dunal)* were procured from 'The Konark herbals and health care'.

Drug: *Bramhyadi* tablet (Figure 1)

Drug preparation date: The final product-Bramhyadi tablet was prepared in the Pharmacy of ITRA, Jamnagar on 01/07/2021(Single batch) Table no.1 shows ingredient with quantity of each drug.

Storage: *Bramhyadi* Tablet was stored in airtight zipped polythene bag at room temperature. It was also given in airtight zipped polythene bag to the patients.

Microbial study duration: every month (01/07/2021 to 13/09/2022)

Table 1: Content of Bramhyadi tablet

Sanskrit name	Botanical name	Part used	Extract	Dose of extract
Brahmi	*Bacopa monnieri* Linn.	Whole plant	Alcohol soluble	150mg
Jatamansi	*Nardostachys jatamansi* Dc.	Root	Alcohol soluble	150mg
Parsik yavani	*Hyoscyamus niger* Linn.	Whole plant	Alcohol soluble	50 mg
Ashwagandha	*Withania somnifera* Dunal	Root	Alcohol soluble	150 mg

Microbial contamination

Two methods were adopted 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

Figure 1: Final product: *Bramhyadi* Tablet

The detailed description and methodology of the procedures are mentioned below and was followed for analysis as mentioned.

1. Smear Examination

A. Wet mount /10% K.O.H. Preparation

Aim: To rule out any mycological findings.

Specimen: *Bramhyadi* tablet

Procedure for Wet Preparation:

Priorly take a clean grease free glass slide and then put selected material on it. Then if needed add distilled water on it. Then observe the slide under the high power (40x) lens with the help of microscope and further report as per the findings.

B. Procedure For 10% KOH Preparation

For preparation of 10% KOH, First take 10% Potassium Hydroxides pellets in clean glass tube with distilled water & mix it well. Now, take clean grease free glass slide and put a drop of specimen and add freshly prepared 10% KOH than cover with grease free cover glass. Then, allow it to react for 15-20 minutes to remove extra debris other than fungal particles and finally observe under high power (40x) lens with the help of microscope. And then report as per findings.

B. Gram's Stain Test

Gramme positive and gramme negative bacteria are separated into two groups using this test. The method is based on microorganisms' capacity to hold onto the colour of the stains used in the gramme stain technique. Gramme positive bacteria are not decoloured because the primary dye is retained by the cell and the bacteria will continue to be purple. Gramme negative bacteria are decoloured by any organic solvent (acetone or Gram's decolourizer). A counterstain effect discovered on Gramme negative bacteria and bacteria will remain pink after the decolonization process. Based on variations in the chemical and physical characteristics of the cell wall, this approach enables bacteria to retain the colour of the stains (Alfred E. Brown, 2001).

Aim: To obviate any bacterial discoveries.

Procedure for Gram's Stain

- To prepare a dry, equally thick preparation (i.e., smear), take a clean, grease-free glass slide.
- By passing the prepared smear over the flame of a Bunsen burner three to four times, the smear was fixed (the fixation kills vegetative form of bacteria and makes them permeable to stain, makes material attach to the surface of the slide and prevents autolytic alterations).
- Cover the fixed, prepared smear with Gram's crystal violet solution and let it sit for the specified amount of time, according to the kit's instructions.

- With tap water, rinse the smear to eliminate extra reagent.
- Cover the smear with Gram's Iodine solution, and then wait the specified amount of time per the kit instructions.
- With tap water, rinse the smear to eliminate extra reagent.
- Holding the slide in the sloped position, pour gram's decolourizer acetone from the bottle onto the smear to decolorize it.
- Gram's decolourizer can be used to decolorize smears by holding the slide in a sloped position and pouring gram's decolourizer acetone from its upper end up to remove the colour of the primary dye (in this case, Gram's Crystal Violet), or as directed by the package instructions.
- Wash the smear with tap water to get rid of the extra acetone.
- Safranin solution should be applied over the smear, and the time specified should pass according to kit instructions.
- With tap water, rinse the smear to eliminate extra reagent.
- Allow to dry, then smear.
- Examine using a lens that is submerged in oil and report your findings.

Culture Study:

Clinical microbiology laboratory generally uses culture methods for isolation of organisms i.e., the lawn/streak culture method.

a. Fungal Culture Method

Following materials were 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)

Use of media: For selective cultivation of pathogenic fungi.

Company: HIMEDIA Laboratories pvt. Ltd.

Required temperature: 37°C

Required time duration: 05 to 07 days.

Procedure for Fungal Culture

- For that analysis, first choose appropriate selective solid media for inoculation purpose. Then dry selective solid media in Hot Air Oven before specimen inoculation and allow it to cool before Specimen inoculation.
- After that Inoculate selective specimen by sterile cotton swab or by Nichrome wire (24 S.W.G. size) loop First sterile loop in Bunsen burner oxidase flame-blue flame and allow it cool after that loop is charged by selected specimen to be cultured. One loopful of the specimen is transferred onto the 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 areal growth and confirm growth by performing different related biochemical reactions and different related staining procedures.
- After that report the isolates.

b. Aerobic Culture Method

Following materials were 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°C

Use of media: for selective cultivation of pathogenic bacteria.

Procedure for Aerobic Culture

- Same procedure till cool dried medium described in fungal culture.
- Selected specimen is inoculated 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 well dried plate

- After that, 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 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 the isolates.

OBSERVATIONS AND RESULTS

Every time sample (preserved rug/ intervention drug) were subjected to the microbiological study from the date of the preparation to the date of last microbiological study.

Table 2: Showing observations of sample preserved at room temperature

Days of investigations after preparation of the sample	Temp.	Humidity	Observations of sample			
			Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
06/07/2021	94°F	49%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
16/08/2021	91°F	55%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
07/09/2021	89°F	73%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
07/10/2021	93°F	61%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
01/11/2021	88°F	26%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
08/12/2021	81°F	39%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
03/01/2022	83°F	46%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
07/02/2022	81°F	39%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
10/03/2022	97°F	22%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
04/04/2022	106°F	15%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
09/05/2022	107°F	16%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
02/06/2022	103°F	13%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
07/07/2022	80°F	96%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
24/08/2022	87°F	66%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
13/09/2022	85°F	85%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated

DISCUSSION

The microbial stability of *Bramhyadi* tablets focused on evaluating the tablet's stability in aspect of microbial quality using baseline microbial diagnostic modalities. The discussion of the study's findings provides insights into the significance of stability studies and microbial assessments in ensuring the safety and efficacy of Ayurvedic formulations.

The study revealed important information regarding the stability of *Bramhyadi* tablets over time. By monitoring the tablet's microbial properties, the researchers were able to determine its shelf life and identify any potential microbial contamination that may occur during storage. This information is crucially helpful for establishing appropriate expiration dates and ensuring that the tablet retains its therapeutic efficacy.

The study also examined the effects of packaging materials on the microbial quality of *Bramhyadi* tablets. As this trail drug was made of dry extract and material was humid, it was stored in airtight plastic bag. But still no microbial contamination was observed throughout study which indicate safe practice of drug storage.

The findings of the stability study have important implications for the safe and effective use of *Bramhyadi* tablets in the management of pre-operative anxiety.

The study highlights the importance of baseline microbial diagnostic modalities in assessing the microbial quality of Ayurvedic formulations. Establishing a baseline microbial profile allows for the detection and monitoring of any deviations or changes that may occur during storage. This information is essential for implementing appropriate quality control measures and ensuring the microbial safety of Ayurvedic products.

Furthermore, the study emphasizes the significance of stability studies in the field of Ayurvedic medicine. Stability studies provide scientific evidence to support the determination of shelf life, recommended storage conditions, and expiration dates of Ayurvedic formulations. These studies contribute to the overall quality assurance and regulatory compliance of Ayurvedic products.

In conclusion, the stability study of *Bramhyadi* tablets in the management of pre-operative anxiety, with respect to baseline microbial diagnostic modalities, provides valuable insights into the tablet's stability and microbial quality. The findings underscore the importance of stability studies and microbial assessments in ensuring the safety, efficacy, and quality of Ayurvedic formulations. By conducting comprehensive stability studies and microbial assessments, manufacturers and healthcare professionals can ensure the integrity of Ayurvedic products and promote patient safety and positive treatment outcomes.

CONCLUSION

Conclusion can be made by this study that Bramhyadi tablet is stable at 107°F maximum temperature & 80°F minimum temperature and stable at changes in humidity (Maximum 85% & minimum 13%). Also, storage in airtight plastic bag was appropriate for 15 months.

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