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STABILITY STUDY OF *ABHAYADI GUTIKA* - WITH RESPECT TO BASELINE MICROBIAL DIAGNOSTIC MODALITIES

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ABSTRACT

Background: Since last couple of decades, market of herbal, herbo-mineral and traditional medicines have grown up by leaps and bounds. The main shortcoming in the propagation of traditional medicines is lack of data pertaining to their stability and shelf life. Hence present study was carried out to observe the stability of *Abhayadi Gutika* with respect to stability against microbial contamination of sample prepared and stored in different climatic conditions and temperatures. **Aim:** To study the stability of *Abhayadi Gutika* and inspect microbial contamination in the finished product at different time intervals and at different climatic conditions (different temperature and humidity set ups). **Materials and Methods:** Samples of *Abhayadi Gutika* were studied to inspect microbial contamination at different climatic conditions. The study was conducted at Microbiology Laboratory, Institute of Teaching and Research in Ayurveda (ITRA), Jamnagar, Gujarat, India. **Observations & Results:** The initial microbiological study of *Abhayadi Gutika* was carried out just before its administration to patients which was seventh day from its preparation. Further studies were carried out at regular time intervals up to 377 days. **Conclusion:** In microbiological study of *Abhayadi Gutika*, growth of micro-

organisms either bacterial or fungal was not found till 377 days from the date of preparation, which shows its intact stability and good shelf life.

KEYWORDS: Stability, Microbial profile, *Abhayadi Gutika*, Climatic conditions.

INTRODUCTION

Stability research gives confirmation of how the nature of a medicinal substance or drug changes over the long duration, impacted by various ecological factors like temperature, humidity and light. It also helps in determining substitution duration for the drug substance and its prescribed storage. Thus one can say stability study is a fundamental aspect as an appraisal of a drug quality.[1] The primary aim of pharmaceutical stability study of a drug is to give fair affirmation that the medications will stay at a proper norm of wellness/quality during the hour of which they are accessible to patients on the lookout and will be appropriate for their utilization before the patient purposes the eventual outcome unit.[2] The specified ideal time duration for consumption of each and every pharmaceutical preparation has been described in classical texts of Ayurvedic Materia Medica like Sharangdhara Samhita. The ancient texts have used the term '*Saviryata Avadhi'* for the time duration for which any medicinal substance's *Virya* (potency) remains unaffected due to environmental/microbial factors.[3] *Gutika* form or tablet preparations of medicine are widely used in Ayurveda pharmaceutical industry by the practitioners of Ayurveda for various ailments. According to Ayurvedic classical texts, *Gutika* or tablet preparations remain potent up to 1 year from their manufacturing date, after which they start degrading gradually, thus losing their efficacy.[4]

The drug *Abhayadi Gutika* studied in present study was prepared in the Department of *Rasashastra* and *Bhaishajya Kalpana*, ITRA, Jamnagar under all possible hygienic conditions. No any preservative was added to the test drug. Drug preparation was finished on 18th of March 2021. Finished product was stored in airtight plastic container at room temperature. In the present study an attempt was made to check stability of *Abhayadi Gutika* with respect to its microbial profile at different climatic conditions and temperature setups at regular intervals for a period of 377 days.

AIM

To study the stability of *Abhayadi Gutika* by inspecting microbial contamination in the finished product at different time intervals and at different climatic conditions (different temperature and humidity set ups).

MATERIALS AND METHODS

Sample of *Abhayadi Gutika* was studied to check microbial contamination at different climatic conditions. The study was conducted at Microbiology Laboratory, Institute of Teaching and Research in Ayurveda (ITRA), Jamnagar, Gujarat, India. Mainly two tests were performed to rule out the existence of any bacteria or fungi in the finished product sample of prepared drug. The initial microbiological study was done on seventh day of preparation, just before its oral administration to the patients. Then samples from same container were subjected to the microbiological study regularly with random intervals during different seasons.

Drug materials

The formulation composition of *Abhayadi Gutika* is summarized at Table 1. *Haritaki*, the chief ingredient of formulation was procured from Pharmacy attached to Institute of Teaching and Research in Ayurveda (ITRA), Jamnagar. Other ingredients viz. *Draksha* (black raisin) and *Sita* (sugar candy) were purchased from local market of Jamnagar and *Madhu* (honey) was obtained from Gujarat State Forest Development Corporation Limited.

Table 1: Ingredients of *Abhayadi Gutika.*

Sr. No.	Drug name	Botanical/English name	Part used	Proportion
1	*Haritaki*	*Terminalia chebula* Retz./Myrobalan	Fruit pericarp	1
2	*Draksha*	*Vitis vinifera Linn.*/Black raisin	Fruit pericarp	1
3	*Sita*	Sugar candy	--	1
4	*Madhu*	Honey	--	1

Preparation Time

The whole process of formulation preparation of *Abhayadi Gutika* was carried out in the Department of *Rasashastra* and *Bhaishajya Kalpana*, Institute of Teaching and Research in Ayurveda (ITRA), Jamnagar, Gujarat, India. The process was completed by following Standard Operating Procedure (SOP) with the utmost care to avoid any sort of contamination.

Date of preparation of drug: 18th March, 2021.

Storage

The finished product, *Abhayadi Gutika* was stored in airtight plastic containers at room temperature in a cool, dark and dry place. Samples of finished product were subjected to

stability study with respect to microbial and fungal contamination at regular time intervals, details of which are cited below.

Microbial Profile

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

1. **Smear Examination**

A. Wet mount /10% KOH Preparation

B. Gram's stain Test

2. **Culture Study**

C. Fungal culture

D. Aerobic culture

The details of the procedures followed are given below.

1. Smear Examination

A. Wet mount /10% KOH Preparation

Aim: To rule out any mycological findings.

Specimen: *Abhayadi Gutika.*

Procedure for Wet Preparation: Take a clean grease free glass slide. Put the selected material. Add some distilled water (q.s.) then cover it with grease free cover glass. Observe under the high power (40x) lens. Note the findings.

Procedure for 10% KOH Preparation: Mix Potassium Hydroxides pellets in distilled water to prepare 10% of the same in clean glass tube. Take a clean grease free glass slide. Put a drop of specimen 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 the high power (40x) lens. Note the findings.

B. Gram's Stain Test

Gram staining is a differential staining technique that differentiates bacteria into two groups: Gram positive and Gram negative. The procedure is based on the ability of microorganisms to retain colour of the stains used during the gram stain procedure. Gram negative bacteria are decolorized by any organic solvent (acetone or Gram's decolourizer) while Gram positive

bacteria are not decolorized as primary dye retained by the cell and bacteria will remain as purple. After decolourization step, a counter stain effect is found on Gram negative bacteria and bacteria will remain pink. The Gram stain procedure enables bacteria to retain colour of the stains, based on the differences in the chemical and physical properties of the cell wall (5).

Aim: To rule out any bacteriological findings.

Specimen: *Abhayadi Gutika.*

Procedure for Gram's Stain: Take clean grease free glass slide to prepare a smear. Fix the prepared smear by passing 3-4 times over the flame of Bunsen burner, then cover the smear with Gram's crystal violet solution and allow it to remain for mentioned time as per kit procedure. Wash off smear to remove excessive reagent with tap water. Cover smear with Gram's Iodine solution and allow it to remain for mentioned time as per kit procedure. Wash off smear to remove excessive reagent with tap water. Decolorize smear with Gram's decolourizer by holding the slide at slope. Wash off smear to remove excess acetone with tap water. Cover smear with Safranin solution and allow it to remain for mentioned time as per kit procedure. Wash 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.

2. Culture Study

C. Fungal culture

The materials collected with sterile cotton swab for inoculation purpose on selected fungal culture media (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:	37^0C
Use of media :	For selective cultivation of pathogenic fungi.

Procedure for Fungal Culture: For inoculation purposes choose the correct selective solid media. Dry selective solid media in a hot air oven before specimen inoculation and allow cooling dried medium before specimen inoculation. 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 to cool than loop is charged with selected specimen to be cultured. One loopful of the specimen is transferred on to the surface of well dried culture media). After the inoculation cycle, incubate the inoculated medium in an inverted position at 37^0C for five to seven to twenty one days in incubator (incubation days are as per growth requirement) in an aerobic atmosphere. After selected incubation period, examine growth by naked eye in form of colony or aerial growth and confirm growth by performing different related biochemical reactions and different related staining procedures. Report the isolates.

D. Aerobic culture method

Respected materials collected with sterile cotton swab for inoculation purpose on selected aerobic culture media (an artificial preparation)

Name of media:	Mac Conkey Agar (MA) and Columbia Blood agar (BA)
Company :	HIMEDIA Laboratories Pvt. Ltd.
Required time duration :	24 to 48 hours
Required temperature:	37^0C
Use of media :	For selective cultivation of pathogenic bacteria

Procedure for Aerobic Culture: Choose appropriate selective solid media for inoculation purpose. Dry selective solid media in a hot air oven before specimen inoculation and allow cooling dried medium before specimen inoculation. Inoculate selective specimen by sterile cotton swab or by Nichrome wire (24 S.W.G. size) loop. After streaking process incubate inoculated medium in inverted position at 37^0C for 18-24 hours in incubator under aerobic or 10% CO2 atmosphere. After selected incubation period examine 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.

OBSERVATIONS AND RESULTS

The initial microbiological study of *Abhayadi Gutika* was done on seventh day of preparation, just before its administration to the patients i.e. first day of drug consumption to rule out any contamination of prepared drug up to consumption of the same. Further study was carried out at regular time intervals up to 377 days i.e. up to last day of consumption of drug. Observations and results obtained in the present study with respect to different humidity levels and temperatures at various stages are depicted in table 2.

Table 2: Microbiological findings at different climatic conditions.

Sr. No.	Study conducted at (No. of days)	Date of Sample given for test	Avg. Temperature ^{0}C (^{0}F)	Humidity (%)	Observations/Findings			
					Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
1	7th Day	25th March 2021	26.1 ^{0}C (79 ^{0}F)	48 %	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
2	36th Day	22nd April 2021	28.8 ^{0}C (83.8 ^{0}F)	56 %	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
3	71st Day	27th May 2021	30.3 ^{0}C (86.6 ^{0}F)	63 %	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
4	98th Day	23rd June 2021	30.4 ^{0}C (86.8 ^{0}F)	69 %	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
5	131st Day	26th July 2021	28.5 ^{0}C (83.4 ^{0}F)	78 %	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
6	161st Day	25th August 2021	27.5 ^{0}C (81.5 ^{0}F)	81 %	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
7	190th Day	23rd September 2021	27.7 ^{0}C (81.9 ^{0}F)	76 %	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
8	222nd Day	25th October 2021	28.7 ^{0}C (83.7 ^{0}F)	57 %	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
9	250th Day	22nd November 2021	26.4 ^{0}C (79.5 ^{0}F)	46 %	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
10	285th Day	27th December 2021	22.3 ^{0}C (72.1 ^{0}F)	47 %	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
11	316th Day	27th January 2022	20.8 ^{0}C (69.5 ^{0}F)	47 %	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated

12	342nd Day	22nd February 2022	22.8 ^{0}C (73 ^{0}F)	47 %	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
13	377th Day	29th March 2022	26.1 ^{0}C (79 ^{0}F)	48 %	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated

DISCUSSION

The unscientific methods of collection, storage, transportation and congenial climatic conditions allow raw materials for herbal formulations prone to fungal & bacterial infestations. The raw materials collected using unscientific methods are commonly exposed to many pathogenic contaminants and are often deteriorated by pathogenic microorganisms during handling and storage.[6] Therefore, lack of regulation for herbal supplements presents potential health risk, largely due to their contamination chances with pathogenic micro-organisms. Other important aspect of drug safety is shelf-life of a drug which is defined as the time period from when the product is produced until the time it is planned to be consumed or used. Several factors are used to determine a product's shelf-life, ranging from organoleptic qualities to microbiological safety. In present study microbiological stability study of *Abhayadi Gutika* was carried out. Samples were selected randomly for the study to rule out any microbiological contamination in entire batch of the finished product. Changes in temperature and humidity of environment were observed and noted during the study period. The city Jamnagar, the region where the drug was prepared and sample was stored is very proximal to sea coast where relative humidity (RH) remains high i.e. minimum 46% & maximum 81% while temperature ranges from minimum 72.1 ^{0}F to maximum 86.8 ^{0}F. High RH can trigger the growth of microbes[7], although RH remained variable throughout the study period, average air quality cannot be considered dry at RH exceeding 40%. Hence wet mount, fungal culture, gram stain and aerobic culture tests were used to study fungal and bacterial contamination in the samples at monthly intervals from 25th March, 2021 to 29th March, 2022. During this study period, no any microbe was isolated as a result of aerobic culture and no any fungal pathogen was isolated as a result of fungal culture (Table 2). Thus at the end of study, it was observed that finished drug samples studied at different time intervals and at different climatic conditions did not show any presence of microbes in them.

CONCLUSION

Stability is usually expressed in terms of serviceable life, which is the time period from when the product is produced until the time it is intended to be consumed or used. In present study microbiological study of *Abhayadi Gutika* showed that there wasn't any growth found of any bacterial or fungal microorganisms till one year from the date of its preparation which shows a good shelf life. The study also proved that quality of the drug was in a standard condition at different climatic conditions viz. relative humidity ranging from 46% to 81% and temperature ranging from 72.1 ^{0}F to 86.8 ^{0}F.

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Conflicts of interest

There are no conflicts of interest.

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