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Exploring the stability of *Mahatiktaka Ghrita*: A comprehensive study on Microbial Integrity

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ABSTRACT

Background: The market for herbal, herbo-mineral, and traditional medicines has grown significantly in recent decades. However, a major challenge to their broader adoption is the limited information on their stability and shelf life. This study was conducted to assess the stability of *Mahatiktaka Ghrita* against microbial contamination when prepared and stored under different climatic conditions and temperatures. **Aim:** To evaluate the stability of *Mahatiktaka Ghrita* and monitor 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 *Mahatiktaka Ghrita* 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 microbiological study of *Mahatiktaka Ghrita* was carried out as per the samples utilized in clinical study. Further studies were carried out at regular time intervals up to 494 days. **Conclusion:** In microbiological study of *Mahatiktaka Ghrita*, growth of microorganisms either bacterial or fungal was not found till 494 days from the date of preparation, which shows its intact stability and good shelf life.

Key words: Stability, Microbial profile, *Mahatiktaka Ghrita*, Climatic conditions

INTRODUCTION

Pharmaceutical stability refers to the ability of a product to maintain its original properties and characteristics within defined limits throughout its storage and use, i.e., its shelf life. It is a critical aspect of drug quality, as any change that occurs after manufacture, which negatively impacts the product's

quality or its suitability for patient use, is a potential concern. This broad area of study addresses various degradation pathways that could affect the integrity of the product over time. It is influenced by numerous factors, including the stability of the active pharmaceutical ingredient(s), the manufacturing process, the dosage form, and the container/closure system. Additionally, environmental conditions encountered during transportation, storage, and handling, as well as the duration between production and use, play significant roles. Environmental factors such as temperature, light, and humidity, alongside chemical processes including oxidation, reduction, hydrolysis, and racemization, can all contribute to drug degradation. Among these environmental variables, temperature stands out as the most critical factor affecting pharmaceutical stability, as it is not easily controlled through packaging alone.^[1]

Thus, it is an essential process aimed at evaluating the changes in the quality, efficacy, and safety of a product

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over time when exposed to various environmental conditions (e.g., temperature, humidity, light). These studies are essential for ensuring that the product retains its intended therapeutic effect, safety profile, and physical/chemical attributes throughout its shelf life. In this context, the current study was designed to assess the stability of *Mahatiktaka Ghrita* in relation to microbial contamination.

Sneha Kalpana is unique pharmaceutical technology of Ayurvedic pharmaceutico- therapeutics and is among those dosage forms which is recommended for administration with various routes and modes of administration. *Goghrita* is considered as best because its ability to assimilate effectively the properties of the ingredients added to it and without losing its own properties (*Samskarasya Anuvartanat*).^[2] Since the cell membrane contains lipids, the lipophilic action of *Ghrita* facilitates transportation of drugs to the target organ and final delivery inside the cell, mitochondria, microsome and nuclear membrane.^[3]

Mahatiktaka Ghrita is first mentioned by Acharya Charaka in *Kushthachikitsa Adhyaya*^[4] and indicated in many diseases like *Kushtha, Raktapitta, Arsha, Visarpa, Amlapitta, Vatarakta, Pandu, Kamala, Jwara, Pama, Unmada, Gulma, Visphota, Kandua, Pidaka, Hridroga, Asrigadara, Gandamala* etc. In context to potency of this formulation, Acharya Charka quoted that, this formulation can cure *Mahavikara*, difficult to get cured even by hundreds of formulations.

Medicated *Ghrita* formulations are extensively utilized by Ayurvedic practitioners for the treatment of various ailments. The term '*Saviryata Avadhi*' refers to the shelf life or potency duration of these preparations, specifically describing the time period during which the potency (*Virya*) of a drug remains effective above a certain threshold. After this period, the potency may gradually decrease, but it does not entirely diminish if the product is stored under proper conditions.^[5] According to classical Ayurvedic texts, the potency of different preparations can remain intact for several months to years, depending on the dosage form.

The *Saviryata Avadhi* of *Ghrita* form is described in Table no. 1 below.

Table 1: *Saviryata Avadhi* of *Ghrita* as per Ayurvedic classics

Form of Preparation	<i>Sharangadhara Samhita</i> ^[6] (13 th century)	<i>Yoga Ratnakara</i> ^[7] (17 th century)	Rule 161(B) of D & C Rules, 1945 ^[8]
<i>Ghrita</i>	16 months (60 days)	12 months (90 days)	2 years

The drug *Mahatiktaka Ghrita* studied in present study was prepared at Dept. of Rasashastra and Bhaishajya Kalpana, Institute of Teaching and Research in Ayurveda (ITRA), Jamnagar under strict hygienic conditions. No any preservative was added to the test drug. Finished product was stored in airtight plastic container at room temperature. In the present study, an attempt was made to check stability of *Mahatiktaka Ghrita* with respect to its microbial profile at different climatic conditions and temperature variations over a span of 494 days, with regular assessments at specified intervals.

AIM

To study the stability of *Mahatiktaka Ghrita* 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

Samples of *Mahatiktaka Ghrita* were 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 samples from the airtight containers were subjected to the microbiological study regularly with random intervals as per different ongoing batch used during clinical study at different seasonal condition.

Drug materials

Cow's ghee and raw drugs were procured from pharmacy attached to Institute of Teaching and Research in Ayurveda (ITRA), Jamnagar. *Amalaki* Fruits (*Emblia officinalis* Gaertn.) were purchased from local market of Jamnagar.

Table 2: Ingredients of Mahatiktaka Ghrita

SN	Ingredients	Botanical Name	Part used	Qty.
Kalka Dravya				
1.	<i>Saptaparna</i>	<i>Alstonia scholaris</i> R.Br.	Dried St. Bk.	1 part
2.	<i>Ativisha</i>	<i>Aconitum heterophyllum</i> Wall.	Dried Rt.	1 part
3.	<i>Aragwadha</i>	<i>Cassia fistula</i> Linn.	Dried Fr. P.	1 part
4.	<i>Katuki</i>	<i>Picrorhiza kurroa</i> Royle ex Benth.	Dried Rt./Rz.	1 part
5.	<i>Patha</i>	<i>Cissampelos paeira</i> Linn.	Dried Rt.	1 part
6.	<i>Musta</i>	<i>Cyperus rotundus</i> Linn.	Dried Rz.	1 part
7.	<i>Ushira</i>	<i>Vetiveria zizanioides</i> Linn.	Dried Rt.	1 part
8.	<i>Haritaki</i>	<i>Terminalia chebula</i> Retz.	Dried Fr.	1 part
9.	<i>Amalaki</i>	<i>Emblia officinalis</i> Gaertn.	Dried Fr.	1 part
10.	<i>Bibhitaki</i>	<i>Terminalia bellirica</i> Roxb.	Dried Fr.	1 part
11.	<i>Patola</i>	<i>Trichosanthes dioica</i> Roxb.	Dried Pl.	1 part
12.	<i>Nimba</i>	<i>Azadirachta indica</i> A. Juss.	Dried St. Bk.	1 part
13.	<i>Parpataka</i>	<i>Fumaria indica</i> Pugsley.	Dried Pl.	1 part

14.	<i>Dhanvayasa</i>	<i>Fagonia cretica</i> Linn.	Dried Pl.	1 part
15.	<i>Shweta Chandana</i>	<i>Santalum album</i> Linn.	Dried Ht. Wd.	1 part
16.	<i>Pippali</i>	<i>Piper longum</i> Linn.	Dried Fr.	1 part
17.	<i>Gajapippali</i>	<i>Scindapsus officinalis</i> schoott.	Dried Fr.	1 part
18.	<i>Padmaka</i>	<i>Prunus cerasoides</i> Linn.	Dried Ht. Wd.	1 part
19.	<i>Haridra</i>	<i>Curcuma longa</i> Linn.	Dried Rz.	1 part
20.	<i>Daruharidra</i>	<i>Berberis aristate</i> Roxb.	Dried St.	1 part
21.	<i>Vacha</i>	<i>Acorus calamus</i> Linn.	Dried Rz.	1 part
22.	<i>Indravaruni</i>	<i>Citrullus colocynthis</i> Schrad.	Dried Fr.	1 part
23.	<i>Shatavari</i>	<i>Asparagus racemosus</i> Willd.	Dried Rt.	1 part
24.	<i>Shwetasariva</i>	<i>Hemidesmus indicus</i> R.Br.	Dried Rt.	1 part
25.	<i>Krushnasariva</i>	<i>Cryptolepis buchanani</i> Roem. & Schult.	Dried Rt.	1 part
26.	<i>Vastakabija</i>	<i>Holarrhena antidiysenterica</i> Wall.	Dried Sd.	1 part
27.	<i>Vasa</i>	<i>Adhatoda vasica</i> Nees.	Dried Pl.	1 part
28.	<i>Murva</i>	<i>Marsdenia tenacissima</i> W. & A.	Dried Rt.	1 part
29.	<i>Amrita</i>	<i>Tinospora cordifolia</i> Willd.	Dried St.	1 part
30.	<i>Kiratatika</i>	<i>Swertia chirata</i> Roxb.	Dried Pl.	1 part

31.	<i>Yashtimadhu</i>	<i>Glycyrrhiza glabra</i> Linn.	Dried Rt.	1 part
32.	<i>Trayamana</i>	<i>Gentiana kurroo</i> Royle.	Dried Pl.	1 part
Sneha Dravya				
33.	<i>Goghrita</i>	Cow's Ghee (Amul brand)	-	128 parts
Drava Dravya				
34.	<i>Amrita</i> <i>Phalarasa</i> (<i>Amalaki</i> <i>Swarasa</i>)	Juice of Indian gooseberry	Fresh Fr.	256 parts
35.	<i>Jala</i>	Potable Water	-	1024 parts

Preparation Time

The whole process of formulation preparation of *Mahatiktaka Ghrita* was carried out in the Dept. of Rasashastra and Bhaishajya Kalpana, Institute of Teaching and Research in Ayurveda (ITRA), Jamnagar, Gujarat, India. The drug was prepared by following Standard Operating process (SOP).

Table 3: Batchwise date of preparation of drug

Batch No.	Drug preparation date
I	23/3/23
II	27/3/23
III	27/4/23
IV	30/4/23
V	01/5/23

Storage

Stability study with respect to microbial and fungal contamination at regular time intervals, details of which are cited below. The finished product, *Mahatiktaka Ghrita* was stored in airtight plastic containers at room temperature in a cool, dark and dry place. Samples of the finished product were analysed

for microbial and fungal contamination at specified time intervals, as outlined below.

Microbial Profile

Microbial contamination was assessed by two methods to check any mycological findings and bacteriological findings. Detail of Microbial profile is mentioned in Table no. 4.

Table 4: Microbial profile

1. Smear Examination	A) 10% KOH Preparation
	B) Gram's stain Test
2. Culture Study	C) Fungal culture
	D) Aerobic culture

1. Smear Examination

A. 10% K.O.H. Preparation:

Aim: To rule out any mycological findings.

Specimen: *Mahatiktaka Ghrita*

Procedure For 10% KOH Preparation

Mix Potassium Hydroxides pellets in distilled water Prepare 10% of the same in clean glass tube & mix well
↓
Take clean grease free glass slide
↓
Put a drop of specimen and add freshly prepared 10% KOH than 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
↓
Note the findings

B. Gram’s stain test:

Aim: To rule out any bacteriological findings.

Specimen: Mahatiktaka Ghrita

Procedure for Gram’s Stain

Take clean grease free glass slide to prepare dry equal thick preparation (i.e. smear)
↓
Fix 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
↓
Wash off smear to remove excessive reagent with tap water
↓
Cover smear with Gram’s Iodine solution and allow remaining for mentioned time as per kit procedure
↓
Wash off smear to remove excessive reagent with tap water
↓
Decolourize smear with Gram’s decolourizer by holding the slide at slope position and pour gram’s decolourizer – acetone from its upper end upto the removal of colour of primary dye (i.e., Gram’s Crystal Violet) or as per kit procedure
↓
Wash off smear to remove excessive reagent with tap water
↓

Cover smear with Safranin solution and allow remaining for mentioned time as per kit procedure
↓
Wash off smear to remove excessive reagent with tap water
↓
Examine under oil immersion lens
↓
Note the findings



Fig. 1: Smear staining Procedure



Fig. 2: Smear staining Procedure

2. Culture Study

C. Fungal culture

The materials collected with sterile cotton swab for inoculation purpose on selected fungal culture media

(an artificial preparation). Details of a fungal culture media used in the study is described in Table no. 5.

Table 5: Fungal Culture

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.



Fig. 3: Sabouraud Dextrose Agar Base (SDA) bottles

Procedure For Fungal Culture

In the clinical microbiology laboratory culture method are employed for isolation of organisms (The lawn / streak culture method is routinely employed)
↓
Choose appropriate selective solid media for inoculation purpose
↓

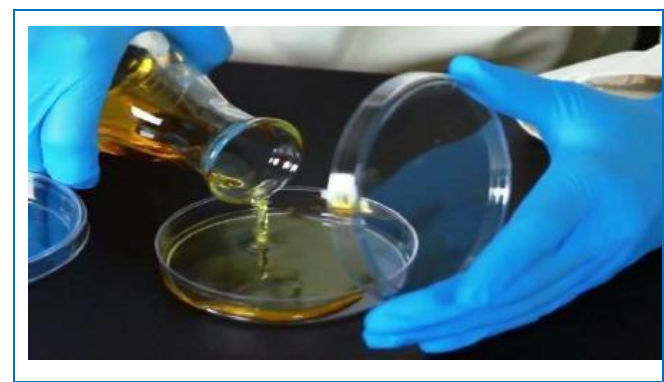
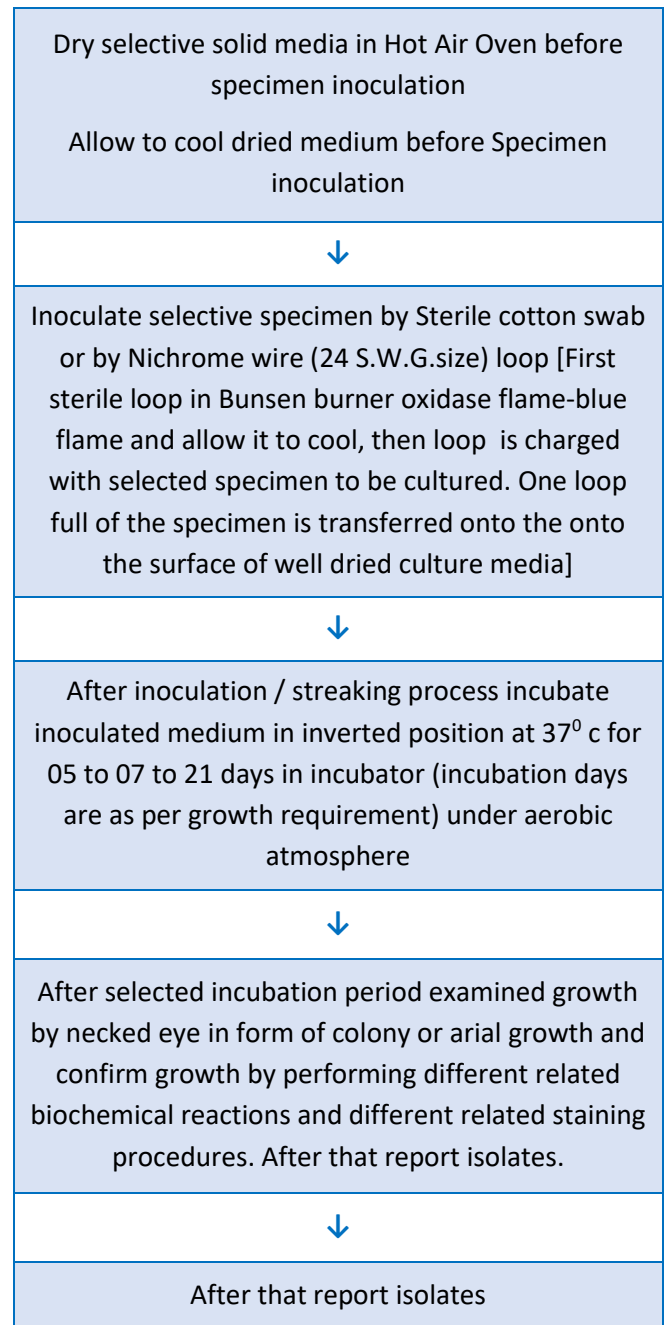


Fig. 4: Procedure for Fungal Culture

D. Aerobic Culture method

Respected materials collected with sterile cotton swab for inoculation purpose on selected aerobic culture media (an artificial preparation). Details of aerobic culture media used in the study is described in Table no. 6.

Table 6: Aerobic Culture

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°C
Use of media	For selective cultivation of pathogenic bacteria



Fig. 5: MacConkey Agar (MA)

Procedure For Aerobic Culture

In the clinical microbiology laboratory culture method are employed for isolation of organism (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 specimen to be cultured. One loopful of the specimen is transferred onto the surface of well dried plate]
↓
After streaking process incubate inoculated medium in inverted position at 37°C for 18-24 hours in incubator under aerobic or 10% CO ₂ atmosphere. After streaking process incubate inoculated medium in inverted position at 37°C for 18-24 hours in incubator under aerobic or 10% CO ₂ 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 isolates



Fig. 6: Procedure for Aerobic culture

OBSERVATIONS AND RESULTS

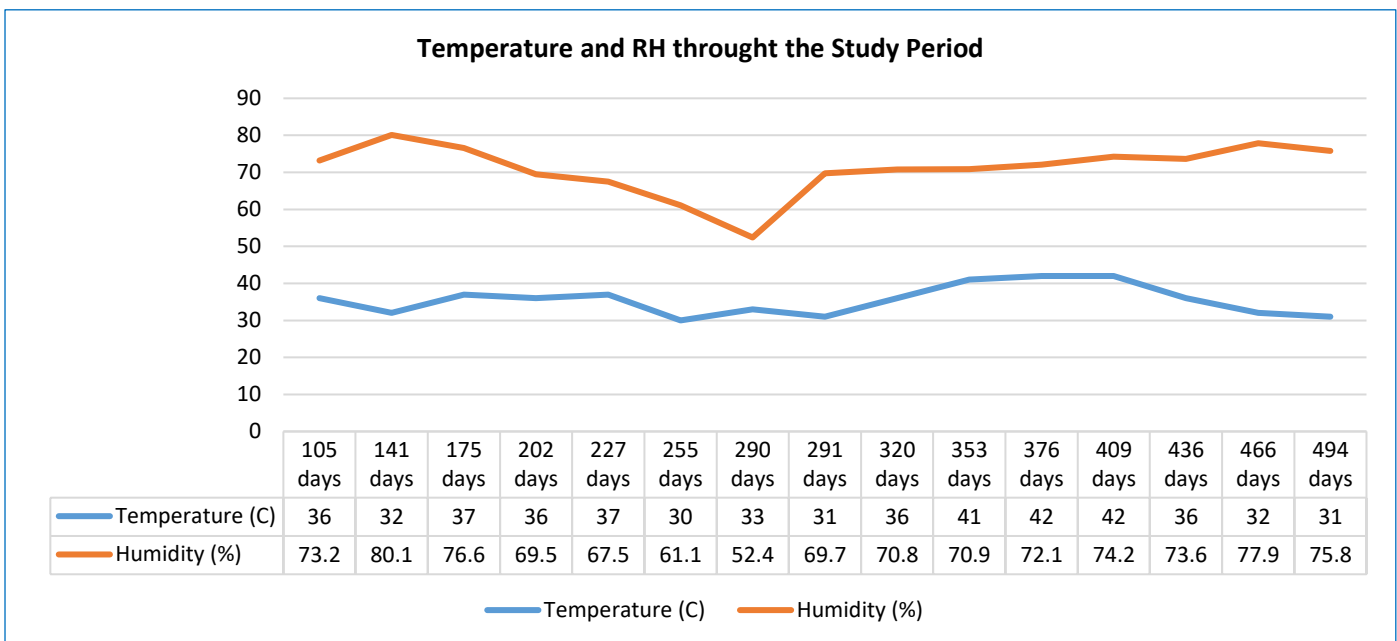
According to batch of prepared drug used in clinical study, at regular time interval prepared samples were

subjected to the microbiological study till completion of the study.

Table 7: Observations and results of Stability tests

SN	Date of Sample given for test	Study conducted at (No. of days)	Avg. Temp.(°C) ^[9]	Avg. Humidity (%) ^[10]	Observations/Findings			
					Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
1.	5/7/23	105 days	36°C	73.2 %	Absence of microorganisms	No organisms isolated	Structure resembling fungal filaments not seen	No fungal pathogen isolated
2.	10/8/23	141 days	32°C	80.1 %	Absence of microorganisms	No organisms isolated	Structure resembling fungal filaments not seen	No fungal pathogen isolated
3.	13/9/23	175 days	37°C	76.6 %	Absence of microorganisms	No organisms isolated	Structure resembling fungal filaments not seen	No fungal pathogen isolated
4.	9/10/23	202 days	36°C	69.5 %	Absence of microorganisms	No organisms isolated	Structure resembling fungal filaments not seen	No fungal pathogen isolated
5.	9/11/23	227 days	37°C	67.5 %	Absence of microorganisms	No organisms isolated	Structure resembling fungal filaments not seen	No fungal pathogen isolated
6.	7/12/23	255 days	30°C	61.1 %	Absence of microorganisms	No organisms isolated	Structure resembling fungal filaments not seen	No fungal pathogen isolated
7.	11/1/24	290 days	33°C	52.4 %	Absence of microorganisms	No organisms isolated	Structure resembling fungal filaments not seen	No fungal pathogen isolated
8.	12/2/24	291 days	31°C	69.7 %	Absence of microorganisms	No organisms isolated	Structure resembling fungal filaments not seen	No fungal pathogen isolated
9.	11/3/24	320 days	36°C	70.8 %	Absence of microorganisms	No organisms isolated	Structure resembling fungal filaments not seen	No fungal pathogen isolated
10.	16/4/24	353 days	41°C	70.9 %	Absence of microorganisms	No organisms isolated	Structure resembling fungal filaments not seen	No fungal pathogen isolated
11.	9/5/24	376 days	42°C	72.1 %	Absence of microorganisms	No organisms isolated	Structure resembling fungal filaments not seen	No fungal pathogen isolated

12.	10/6/24	409 days	42°C	74.2 %	Absence of microorganisms	No organisms isolated	Structure resembling fungal filaments not seen	No fungal pathogen isolated
13.	9/7/24	436 days	36°C	73.6 %	Absence of microorganisms	No organisms isolated	Structure resembling fungal filaments not seen	No fungal pathogen isolated
14.	8/8/24	466 days	32°C	77.9 %	Absence of microorganisms	No organisms isolated	Structure resembling fungal filaments not seen	No fungal pathogen isolated
15.	5/9/24	494 days	31°C	75.8 %	Absence of microorganisms	No organisms isolated	Structure resembling fungal filaments not seen	No fungal pathogen isolated



Graph 1: Timeline of Temperature and RH throughout the Study period.

DISCUSSION

Pharmaceutical stability studies are crucial for regulatory approval, ensuring that drugs maintain their intended quality and safety throughout their shelf-life, which is defined as the period from production to intended use. In the case of herbal formulations, the unscientific collection, storage, and transportation methods expose raw materials to contamination by pathogenic microorganisms, such as fungi and bacteria, which can lead to product deterioration.^[11] The lack of regulation in the herbal supplement industry further increases the risk of microbial

contamination, posing potential health hazards to consumers. A drug's shelf-life is determined by various factors, including organoleptic properties to microbiological safety, to guarantee that the product remains safe and effective for use before expiration.

In present study microbiological stability assessment of *Mahatiktaka Ghrita* was conducted. Samples were selected based on those used in the clinical study to detect any microbiological contamination in the entire batch of the finished product. Variations in temperature and humidity of the environment were monitored and recorded throughout the study period.

Mahatiktaka Ghrita, prepared and stored at room temperature, ranging from a minimum of 30°C to a maximum of 42°C, conditions favourable for bacterial growth-remarkably remained free of microbes. Located in the coastal region of Jamnagar, which is known for its consistently high relative humidity throughout the year, it defied expectations. Despite relative humidity levels varying from a low of 52.4% on January 11, 2024 to a high of 80.1% on August 10, 2023 as shown in Table no. 7, no bacterial or fungal growth was detected throughout the duration of the study.

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 6). 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. While the general concept of stability for both Ayurvedic and modern medicines remains the same, the specific parameters used to assess stability may differ depending on the product.

CONCLUSION

Stability study is an essential part of product development and regulatory processes, aimed at ensuring the quality, safety, and efficacy of products over time. In the current study, the microbiological analysis of *Mahatiktaka Ghrita* demonstrated no bacterial or fungal growth up to sixteen months from its preparation, indicating a good shelf life. Additionally, the study confirmed that the drug's quality remained within standard parameters under various climatic conditions, with relative humidity ranging from 52.4 % to 80.1 % and temperatures between 30°C and 42°C.

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