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STABILITY STUDY OF *MUSTADI GHANA VATI* WITH RESPECT TO VARIOUS MICROBIAL DIAGNOSTIC MODALITIES

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ABSTRACT

Background: In last few decades, market of herbal and traditional medicines have grown up in leaps and bounds, but majority of traditional medicines are lacking in its data of *Saviryatavadhi* (shelf-life period). Hence the present study was carried out to evaluate the stability of *Mustadi Ghana Vati* with respect to microbial contamination and fungal growth. **Material and method:** *Mustadi Ghana Vati* was prepared in the pharmacy, ITRA and stored in food grade air-tight plastic containers, in a well-ventilated, well lighted space at room temperature. A baseline microbial profile was studied at regular interval of 1 month for total 13 months. Every time, the sample was subjected to microbiological studies like smear examination with wet mount - 10% K.O.H. preparation and Gram's stain; fungal culture; and aerobic culture at Microbiology Laboratory, ITRA, Jamnagar. **Results**: In this study, no any growth of micro-organisms (bacterial or fungal) was found till 2nd March 2022 i.e. for 13months from the date of preparation. **Conclusion:** Hence this study shows that *Mustadi Ghana Vati* can remain stable in various climatic conditions for a minimum of 13 months and remain free from any microbial contamination, which indicate that the manufacturing and storage practices adopted in this study was up to the mark of Good Manufacturing Practice (GMP) and could be used as quality standards by future researchers and drug manufacturers.

Key words- *Mustadi Ghana Vati*, drug stability, microbial contamination, growth of microbes

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INTRODUCTION:

Stability of a pharmaceutical product refers to the capability of a particular formulation in a specific container or closure system, to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications at a defined storage condition. Stability research provides proof of how the quality of a drug substance or product changes over time, affected by a number of environmental factors such as temperature, humidity, and light, as well as determining the substitution duration for the drug substance or product and prescribed storage. Hence, one can say that, stability study proves to be a necessary tool for assessment of the quality of any product [1]. The main purpose of pharmaceutical stability testing is to provide fair assurance that, the drugs will remain at an appropriate standard of fitness / quality during the time of which it is available to patients in the market and will be suitable for their consumption [2]. Ayurvedic Formulary of India (AFI) has also provided the time from the date of manufacture till the time they should be consumed; for better results. In the Ayurvedic literatures, the word "*Saviryata Avadhi*" (shelf life) is stated in the sense of the time interval in which any drug's *Virya* (potency) remains unaffected due to environmental or microbial degradation. *Ghana Vati* (tablet) preparations are widely and largely used in pharmacies as well as by practitioners of Ayurveda for various ailments. According to Ayurveda classics, *Ghana Vati* preparations remain potent up to 1 year [3], [4], after which they start losing their efficacy, whereas the shelf-life of *Ghana Vati* has been mentioned as 3 years in Gazette of India [5].

Acharya Charaka has mentioned *Mustadi Kashaya* (decoction) for the treatment of *Santarpana Janya Vyadhi* (diseases due to over nutrition) [6]. To make this decoction palatable and to make it easily dispensable, it was converted to *Ghana Vati* (reduction of that decoction through heat to get the solid end product) formulation. This drug is being widely used by many Ayurveda physicians to manage diseases like *Sthaulya* (overweight and obesity), *Prameha* (diabetes), dyslipidaemia [7], [8], etc. but data on stability study of this formulation is not available till date.

Hence the present study was aimed to evaluate the stability of *Mustadi Ghana Vati* (MGV) with respect to microbial contamination and fungal growth

MATERIALS AND METHODS:

In this study, the raw drugs were procured and *Mustadi Ghana Vati* (MGV) was prepared in the pharmacy, ITRA. (Table 1)

Table 1: Ingredients of *Mustadi Ghana Vati*

Sr. No.	Ingredients	Botanical/ English name	Part Used	Ratio
1	*Musta*	*Cyperus rotundus* Linn.	Rhizome	1part
2	*Aragwadha*	*Cassia fistula* Linn.	Pulp of fruits	1part
3	*Patha*	*Cissampelos pareira* Linn.	Root	1part
4	*Haritaki*	*Terminalia chebula* Linn.	Fruit pericarp	1part
5	*Bibhitaki*	*Terminalia belerica* Linn.	Fruit pericarp	1part
6	*Aamalaki*	*Embelica officinalis* Gaertn.	Fruit pericarp	1part
7	*Devadaru*	*Cedrus deodara* Roxb.	Heart wood	1part
8	*Gokshura*	*Tribulus terrestris* Linn.	Root	1part
9	*Khadira*	*Acacia catechu* Linn.	Heart wood	1part
10	*Nimba*	*Azadirachta indica* A.Juss.	Stem bark	1part
11	*Haridra*	*Curcuma longa* Linn.	Dried rhizomes	1part
12	*Daruharidra*	*Berberis aristata* DC.	Dried stem	1part
13	*Kutaja*	*Holarrhena antidysenterica (*Roth) A.	Stem bark	1part

Method of preparation of MGV: The authenticated crude drugs were crushed to a coarse powder separately (180 µm IS sieve) and then mixed thoroughly with equal proportion of each ingredients (1part each). 16 parts of water was added to it, in a stainless steel container and then continuous mild heat was supplied until it was reduced to 1/8th of its initial quantity. During the heating process, continuous stirring was done to facilitate the evaporation and to avoid any deterioration due to burning of materials. After a desirable reduction in volume was achieved, the *Kwatha* (decoction) was filtered through a single folded cotton cloth and collected in a separate vessel [9].

Kwatha (decoction) was boiled again over slow fire on a gas stove, maintaining the temperature between 90°C - 95°C till a semisolid consistency was obtained. As the water evaporated, the viscosity of the extract increased [10]. Then, the material was mixed with the fine *Churnas* (powders) of *Mustadi Kashaya* (up to 10 % of extract) further forming a solid mass. The solid mass (*Ghana*) was forced through a 16 number sieve and granules were prepared and then, rounded *Vatis* (tablets) were prepared from it with a target weight of 500mg and dried in a tray-dryer at a temperature not exceeding 60 °C for 10 to 12 h [11], [12].

MGV was prepared on 17th Feb 2021. Subsequently, it was stored in food grade air-tight plastic containers, in a well-ventilated, well lighted space in the department, at room temperature.

The room temperature was checked using a wall mounted room thermometer each time, during the testing of the sample. Humidity on the day of testing of sample was noted from the authentic website https://www.timeanddate.com/weather/india/jamnagar for regional weather report.

First time, sample of MGV was evaluated for microbial contamination after 15 days of its preparation. Subsequently, samples from the same container were subjected to microbiological study every month and hence samples had faced various seasons till 2nd March 2022 i.e. for a period of 13 months. This stability study was carried out at Microbiology Laboratory, ITRA, Jamnagar. Four tests were performed to rule out the existence of any bacteria or fungi in MGV.

1. Smear examination- Wet mount - 10% K.O.H. preparation (to rule out any mycological findings) and
2. Smear examination -Gram's stain (to rule out any bacteriological findings)
3. Fungal culture
4. Aerobic culture

The details of the procedures followed are as given below.

1. **Smear examination- Wet mount /10% K.O.H. Preparation:** Potassium Hydroxide pellets were taken in distilled water to prepare 10% of the same in a clean glass tube & mixed well. Later, a clean grease free glass slide was taken. A drop of the specimen was put over it and freshly prepared 10% KOH solution was added, which was then covered with a grease free cover glass. This was allowed to react for 15-20 minutes to remove extra debris other than the fungal particles and later observed under a high power (40x) lens. The report was prepared as per the findings
2. **Smear examination - Gram's stain test:** A clean glass slide was taken to prepare a dry thick smear. The smear was fixed by heating it over the flame. Then Gram's crystal violet solution was put on the fixed smear & left for a certain time as mentioned on the kit. The smear was later washed in running tap water to get rid of excessive Crystal Violet solution. Then, Gram's Iodine solution was added to the smear and left for a certain time period as mentioned on the kit. Later, the smear was washed in running tap water to get rid of the excessive solution. Then, stream gram's decolourizer (acetone) was put on the slide so as to remove the colour of the Gram's crystal violet or as per the kit; the smear was washed in running tap water to get rid of the excessive acetone. The smear was counter stained with Safranin solution & left for a certain time period as mentioned on the kit. Then, the smear was

washed in running tap water to get rid of the excessive solution. The smear was left for drying. It was inspected under an oil immersion lens and the findings were recorded [13], [14].

3.

Fig. 1: Smear staining procedure:

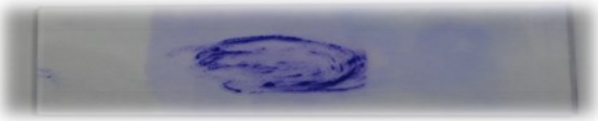


Fig. 2: Stained smear ready for examination

4. **Fungal culture method:** Requisite materials were collected with a sterile cotton swab for inoculation purpose on a selected fungal culture media (i.e. an artificial preparation).

Name of media: Sabouraud Dextrose Agar (SDA) Base -Modified (Dextrose Agar Base, Emmons)

Company: HIMEDIA Laboratories Pvt. Ltd.

Required time duration: 05-07 days

Required temperature: 37 ºC

Use of media: For selective cultivation of pathogenic fungi.

Fig. 3 - Sabouraud Dextrose Agar Base

Procedure for fungal culture: Sabouraud Dextrose Agar (SDA) Base was taken for inoculation purpose. This media was dried in hot air oven before specimen inoculation. The specimen on this media was infused by sterile cotton swab/ Nichrome wire (24 S.W.G. size) loop. The infused media was incubated in inverted position at 37^0c under aerobic atmosphere for 5-7-21 days (as per requirement). A colony or arial growth was seen by the naked eye after the incubation period. The growth was verified by related biochemical reactions & staining procedures and later, a report was prepared as per the findings.

5. **Aerobic culture method:** Necessary materials were collected with sterile cotton swab for inoculation purpose on an aerobic culture media (i.e. an artificial preparation)

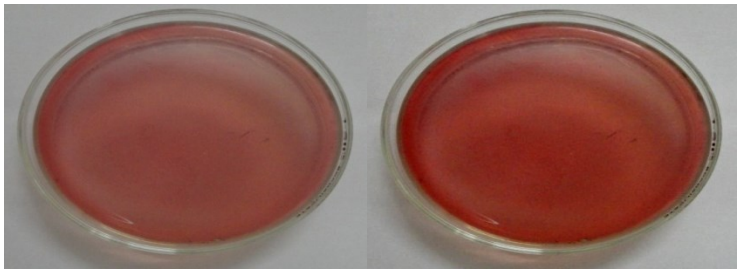
   Name of media: MacConkey Agar (MA) and Columbia Blood Agar (BA) Base.

   Company: HIMEDIA Laboratories Pvt. Ltd.

   Required time duration: 24-48 hours

   Required temperature: 37 ºC

   Use of media: For selective cultivation of pathogenic bacteria.



A **B**

Fig. 4. A: Aerobic culture media (MA), Fig. 4. B: Aerobic culture media (BA)

Procedure for Aerobic culture: The selective solid media was taken. This media was dried in hot air oven & let to cool down. The sample on this media was infused by applying the four flame methods by Nichrome wire (24 S.W.G. size) loop (streaking process). This media was incubated in inverted position at 37^0c. The growth could be seen by naked eye after incubation period. The growth was verified by related biochemical reactions & staining procedures and later, a report was prepared [15].

Observations and results:

In this study, no any growth of micro-organisms (bacterial or fungal) was found from 03/03/2021 till 02/03/2022 i.e. for 13months. (Table 2)

Table 2: Results of microbiological study of *Mustadi Ghana Vati* (Drug preparation date: 17th Feb 2021)

Sr. No.	Date when sample was given	Temp. & Humidity	Observations			
			Gram's stain	Aerobic culture	Wet mount/ 10% KOH preparation	Fungal culture
1.	03/03/2021	23^0C 22%	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
2.	12/04/2021	27^0C 33%	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
3	19/05/2021	34^0C 46%	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
4	30/06/2021	30^0C 57%	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
5	22/07/2021	31^0C 70%	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
6	18/08/2021	30^0C 59%	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
7	27/09/2021	32^0C 70%	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
8	27/10/2021	32^0C 37%	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated

9	24/11/2021	19^0C 36%	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
10	13/12/2021	21^0C 33%	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
11	19/01/2022	21^0C 28%	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
12	07/02/2022	20^0C 36%	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
13	02/03/2022	24% 29^0C	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated

DISCUSSION:

Microbial contamination should be avoided to maintain the drug stability. There are many factors like temperature, humidity, moisture etc. which can have an impact on microbial growth in a drug. These factors should be taken care by adopting GMP (Good Manufacturing Practice), for better stability.

Mustadi Ghana Vati was prepared and stored at room temperature. Optimum temperature for microbial growth is the temperature at which microbes multiplies, this optimum temperature for Psychrophilic bacteria (cold loving bacteria) is 15-20^0C while for Mesophilic bacteria (middle loving) is 30- 37^0C and for Thermophilic bacteria (heat loving) is 50-60^0C [16]. In this study, as mentioned in table 2, temperature setups ranged from minimum 19^0C to maximum 34^0C, which proved as standard temperature for various types of bacteria to overgrow, but there were no any microbes isolated till 13 months of drug preservation after preparation. High Relative Humidity (RH) allows the growth of microbes [17]. The Jamnagar region, where this drug was prepared and stored in present study is proximal to the sea coast, where the RH remains high in all the seasons

of the year. As shown in Table 2, highest RH observed was 70% in the month of July and September, while lowest humidity was 22% in month of March. Although RH remained variable throughout the study period, microbial growth was not observed during this study. This indicates that manufacturing and storage procedures adopted in this study was up to the mark.

Moisture content is one of the main causative factors in drug deterioration and it also acts as an enzymatic activator which slowly decomposes the drug resulting in its degradation [18]. In this study, MGV was prepared through heating method, in which decoction was reduced to solid end product, due to which, moisture content was reduced, which might have played an important role in its long stability.

Limitations: Due to monitory restrictions and stipulated time frame, this study was carried out till 13 months only, which has given only a minimum idea of the stability period of MGV. A long term stability study or an accelerated stability study is required to get the actual idea about shelf-life of the MGV. These findings can be generalized for MGV, which is prepared from the ingredients collected from same geographical regions; because changes in the concentration of phytochemicals of herbs are very common with a change in the geographical region.

CONCLUSION:

Hence this study shows that *Mustadi Ghana Vati* can remain stable in various climatic conditions for a minimum of 13 months and remain free from any microbial contamination, which indicate that the manufacturing and storage practices adopted in this study was up to the mark of Good Manufacturing Practice (GMP) and could be used as quality standards by future researchers and drug manufacturers

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CONFLICT OF INTEREST:

There were no any conflicts of interest

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