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Stability study of *Amalakibhavita nisha* used in the management of type 2 diabetes (*Madhumeha*) - with respect to baseline microbial diagnostic modalities

Vasundhara Sharma, Sachin Kumar Sharma, MS Cholera, DH Pandya and Anup Thakar

Abstract
Background: Diabetes mellitus is a clinical syndrome characterised by hyperglycaemia caused by absolute or relative deficiency of insulin. In *Ayurveda* disease type 2 diabetes can be correlated with *Madhumeha*. *Madhumeha* (Type 2 Diabetes) is caused by the involvement of all *Doshas* and ten *Dushya*. *Nisha Amalaki* is one of the *Ayurvedic* formulation widely used in the management of *Madhumeha* (Diabetes Mellitus). In the present study 7 *Bhavna* (trituration) of *Amlaki* (*Emblica officinalis*) *Swaras* (juice) have been given to *Haridra Choorna* (*Curcuma longa*).
Aim: To carried out study of *Amalakibhavita Nisha* with respect to its stability against microbial contamination
Materials and Methods: Sample of *Amalakibhavita Nisha* was prepared and studied at regular time intervals to check microbial contamination. *Vati* was stored in plastic container during different climatic conditions were studied at regular intervals of 1 month for a period of 15 months to analysis Mycological findings and presence of bacteriological findings by Wet mount preparation and Gram stain test respectively.
Results: Sample was subjected to the microbiological study from the date of the preparation (17 January 2017) to the date of last microbiological study (30 April 2018). No any contaminations were found in microbiological study.
Discussion: This study was carried out to observe the stability study of *Amalakibhavita Nisha* store in different climatic conditions and temperature with respect to Microbial Contamination of prepared sample. Thus a baseline Microbial profile was studied at regular interval of 1 month for total 15 month (i.e. time for consumption of prepared drug). At the end of study sample was not showed presence of any Microbes.
Conclusion: At the end of study *Vati* container has not present of microbes even in different climate and temperature, after 15 months of preparation sample, Hence in present study the stability test of *Amalakibhavita Nisha* with respect to microbiological findings was negative at room temperature, warm and cold, dry and humid conditions.

Keywords: *Amalakibhavita Nisha*, climate conditions, microbial profile, stability, type 2 diabetes

1. Introduction
Diabetes mellitus is a clinical syndrome characterised by hyperglycaemia caused by absolute or relative deficiency of insulin. Hyperglycaemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels [1]. In *Ayurveda* disease diabetes mellitus can be correlated with *Prameha/Madhumeha*. It is *Tridosha* jain origin with involvement of ten *Dushya*. *Nisha Amalaki* is very effective formulation mentioned in *Ashtanga Hridaya* [2]. *Amalaki* (*Emblica officinalis*), family Euphorbiacae, is sweet, sour, astringent, pungent and bitter in taste. The other ingredient is *Haridra* (*Curcuma longa*), family Zingebearaceae, it is *tridoshsamak* [3]. *Haridra* due to bitter taste pacifies *Pitta* and because of hot potency pacifies *Vata and Kaphadosha* [4]. Till date no scientific work has been reported on effect of *Bhavna* on *Haridra choorna* by *Amlaki swaras.*
According to the World Health Organization (WHO) report, India today heads the world with over 32 million diabetic patients and this number is projected to increase to 79.4 million by the year 2030 [5]. Recent surveys indicate that diabetes now affects a staggering 10-16% of urban population [6] and 5-8% of rural population in India [7].

Ayurvedic management of *Madhumeha* (Type 2 Diabetes) aims not only to achieve a good glycaemic control but also to treat the root cause of disease and its prevention. For the first time the research work carried out for its authentication and microbial profile. The drug was prepared in pharmacy of Gujarat Ayurved University, Jamnagar by adopting standard operative procedure for *Vati* formation. No any preservative was added to the test drug. Drug preparation was finished on 17 January 2017. Finished product was stored in airtight plastic containers at room temperature. Thus in the present study on attempt was taken to check stability of *Vati* with respect to its Microbial profile at different climatic conditions and temperature setups at regular interval for a period of 13 months.

Aim

To study the stability and to check microbial contamination of *Amalakibhavita Nisha* at different time interval, at different climatic conditions, temperature and humidity set ups.

Materials and Methods

Sample of *Amalakibhavita Nisha* was prepared (stored at room temperature) and finished product studied to check microbial contamination at regular intervals of 1 month for a period of 13 months (upto drug used). Microbiological study has been carried out in Microbiology Laboratory, IPGT & RA, Jamnagar. Mainly 02 studies have been carried out to rule out that presence of any bacteria or fungi in the prepared drug as a final finished product.

The initial microbiological study was done on 86th day of preparation. Then samples from same container were subjected to the microbiological study regularly with intervals of 1 month during different seasons.

Drug material

All the raw drugs were obtained from Pharmacy of Gujarat Ayurved University, Jamnagar. The ingredients and the part used are given in table 1.

Table 1: Ingredients of *Amalakibhavit Nisha (A.H.Utt.*40/48)

Drug	Latin name	Part to be used
Nisha (*Haridra*)	*Curcuma longa Linn*	Rhizome
Amalaki	*Embelica officinalis Gaertn.*	Fruit juice

*Seven *Bhavna* of *Amlaki* swaras have been given to *Nisha* (*Haridra) Choorna.* After that *Vati* were prepared.

Date of drug preparation: 17 January 2017

Storage

Finished product of *Amalakibhavit Nisha* was stored in air-tight food grade, plastic containers, stored in the open light area in the department at room temperature. Clean and dry stainless steel spoon was used to take medicine.

Microbial profile

Microbial contamination was assessed by two methods 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

The details of the procedures followed are given below

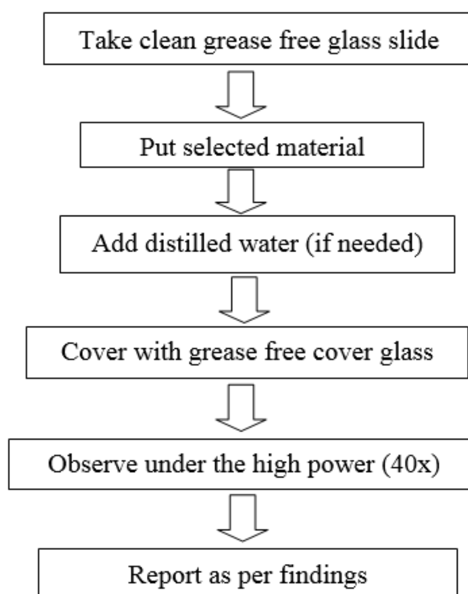
1. Smear Examination

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

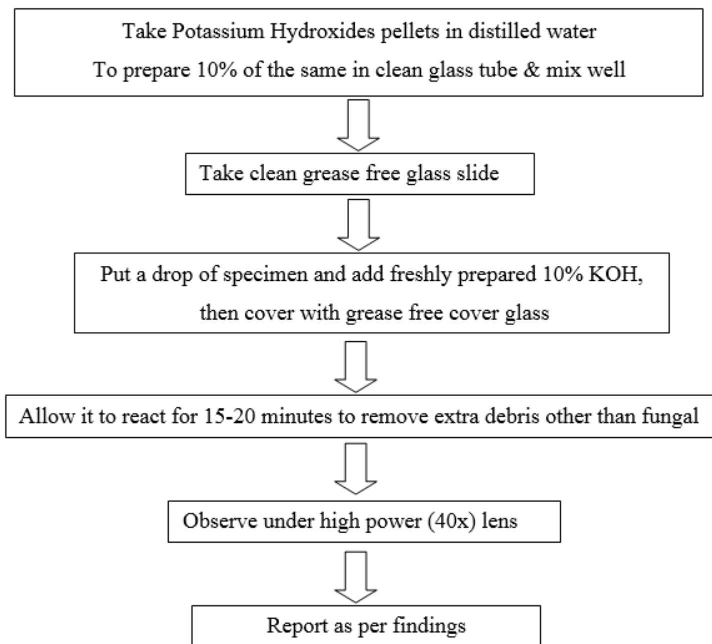
Aim: To rule out any mycological findings.

Specimen: *Amalakibhavit Nisha*

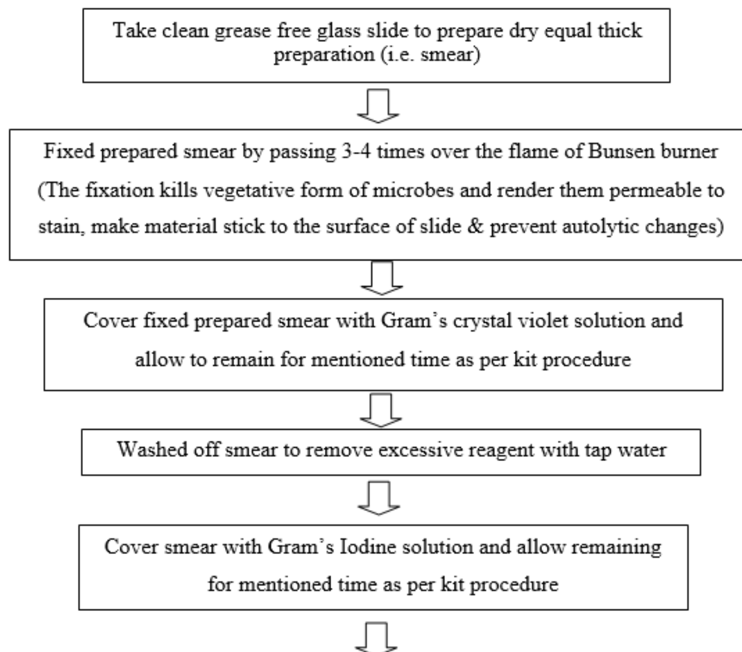
Procedure for wet preparation



Procedure for 10% KOH preparation



Procedure for gram's stain



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 decolorizer) while Gram positive bacteria are not decolorized as primary dye retained by the cell and bacteria will remain as purple. After decolorization step, a counter stain effect found on Gram negative bacteria and bacteria will remain pink. The Gram stain procedure enables bacteria to retain color of the stains, based on the differences in the chemical and physical properties of the cell wall (Alfred E Brown, 2001) [8].

Aim: To rule out any bacteriological findings.

Specimen: *Amalakibhavit Nisha*

Procedure for gram's stain

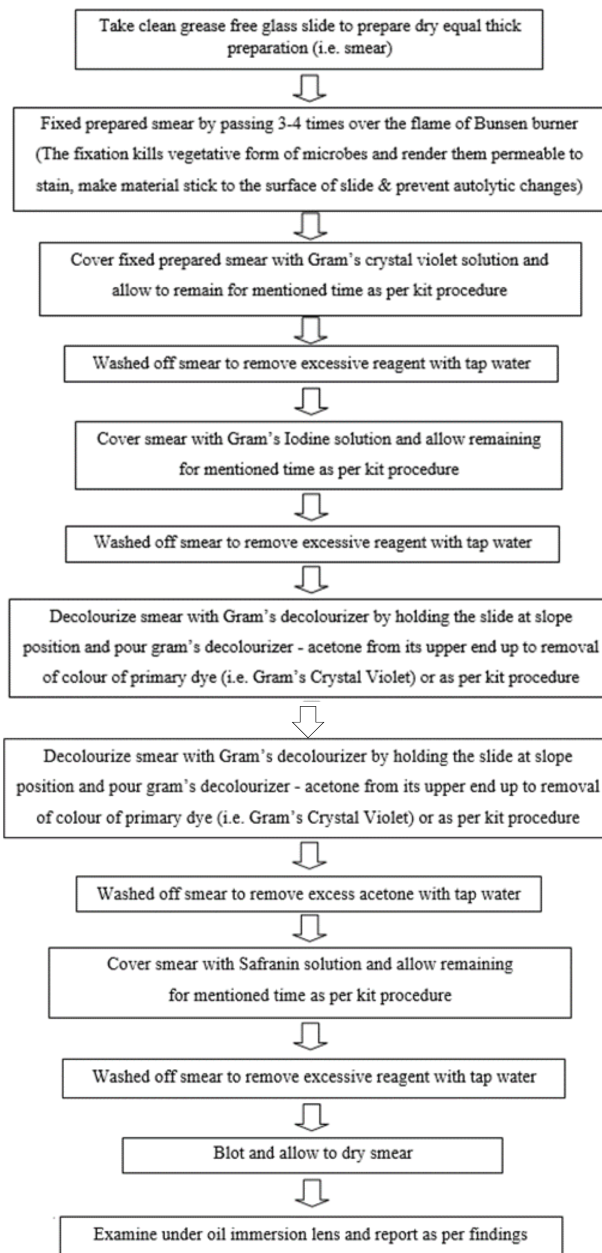


Fig 1-2: Smear staining Procedure

Fig 3: Stained smear ready for examination

Culture Study

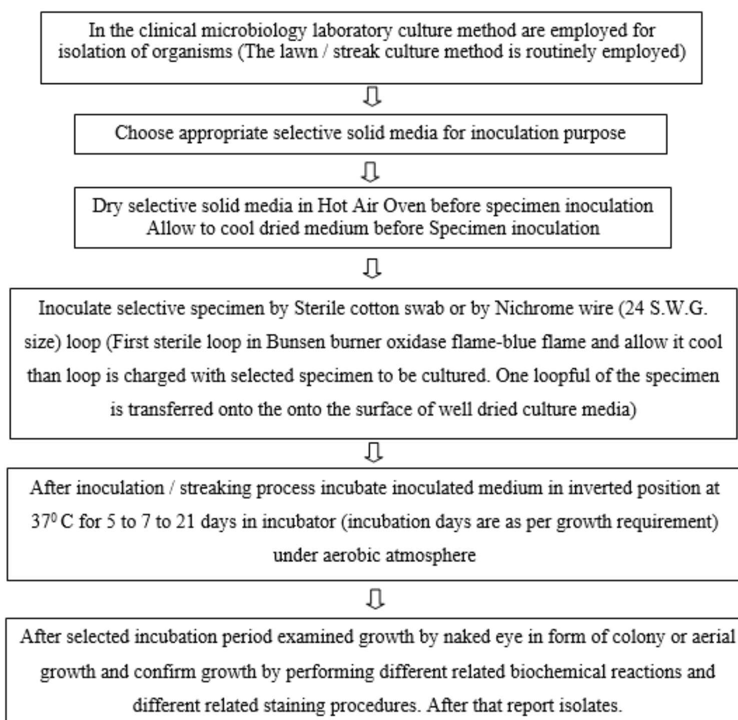
Fungal culture method

Respected materials collected with sterile cotton swab for inoculation purpose on selected fungal culture media (i.e. an artificial preparation).
Name of media: 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.

Fig 4: Sabouraud Dextrose Agar Base (SDA)

Procedure for fungal culture



Respected materials 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 ºC
Use of media: for selective cultivation of pathogenic bacteria.

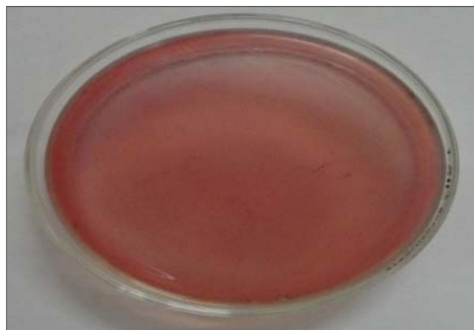


Fig 5: Aerobic culture media (MA)

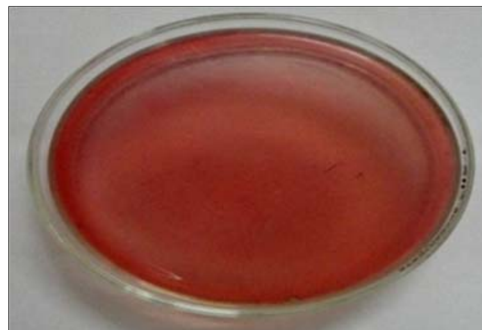
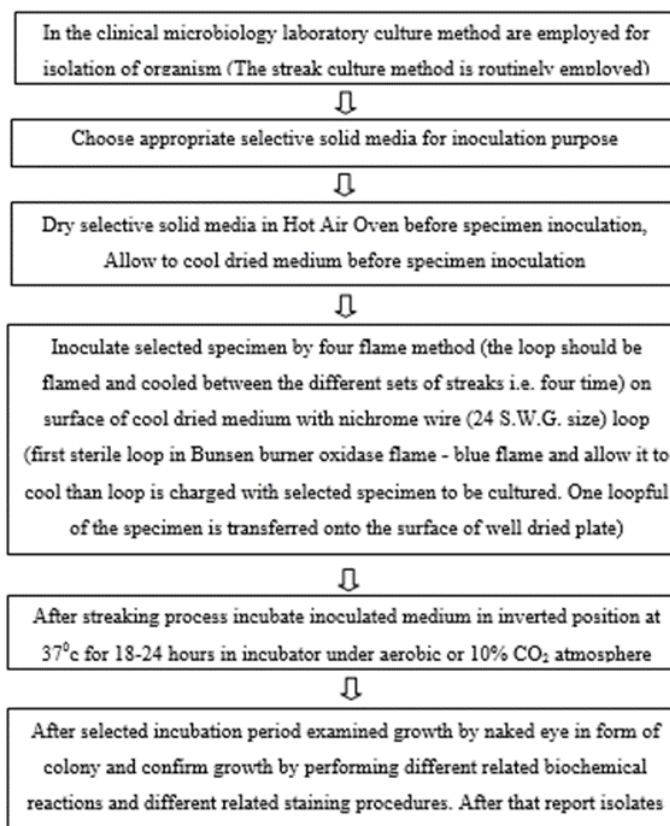


Fig 6: Aerobic culture media (BA)

Procedure for aerobic culture



Observations & Results

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 are shown in table 2.

Table 2: Showing observations of sample preserved at room temperature

S. N.	Months of investigations	Temper-ature	Humidity	Observation of Both samples			
				Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
1.	3rd month (13/04/2017)	41ºC	30%	Microorga-nisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
2.	4th month (17/05/2017)	43ºC	35%	Microorga-nisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
3.	5th month (13/06/2017)	41ºC	38%	Microorga-nisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
4.	6th month (18/07/2017)	32ºC	74%	Microorga-nisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
5.	7th month (23/08/2017)	30ºC	80%	Microorga-nisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
6.	8th month (21/09/2017)	33ºC	69%	Microorga-nisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
7.	9th month (12/10/2017)	33ºC	61%	Microorga-nisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
8.	10th month (16/11/2017)	34ºC	30%	Microorga-nisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
9.	11th month (20/12/2017)	29ºC	24%	Microorga-nisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
10	12th month (16/01/2018)	34ºC	36%	Microorga-nisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
11	13th month (15/02/2018)	32ºC	24%	Microorga-nisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
12	14th month (26/03/2018)	42ºC	28%	Microorga-nisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
13	15th month (30/04/2018)	42ºC	32%	Microorga-nisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated

Discussion

Ayurveda is widely used in management of *Madhumeha* (Type 2 Diabetes). In the present study *Amalakibhavita Nisha* has been used in the management of *Madhumeha.* In present study, it has shown very good and encouraging results in management of *Madhumeha* (Type 2 Diabetes). Hence the present Study was carried out to observe the stability study of *Amalakibhavita Nisha* with respect to Microbial Contamination in different climatic and temperature conditions. Thus a baseline Microbial profile was studied at regular interval of 1 month for total 13 months for *Amalakibhavita Nisha*. At the end of study it was found that sample has not shown presence of any Microbes.

Stability is usually expressed in term of shelf-life, which is the time period from when the product is produced until the time it is intended to be consumed or used. Microorganisms needs water, humidity and temperature at suitable environmental conditions to develop in any media, surface or article. The factors affecting stability of prepared drug are categorized under intrinsic and extrinsic factor (FDA report 2001). Intrinsic factors include moisture content, acidity, nutrient content, biological structure, redox potential, naturally occurring and added antimicrobials. Extrinsic factors include types of packaging, effect of time/temperature on microbial growth, storage/holding conditions and processing steps (FDA report 2001). The region where the drug was prepared and sample was stored was very proximal to sea coast, this area has longest sea shore and maximum number of sea ports, so relative humidity (RH) remains high in all the seasons of the year. Highest RH observed was 80% in month of August while lowest relative humidity was 24% observed in month of February (as shown in Table 2). High RH may allow the growth of microbes [9], RH remain variable during whole study period. Wet mount, fungal culture, gram stain and aerobic culture tests were used to rule out any fungal and bacterial contamination in the sample of monthly interval from 13th April 2017 to 30th April 2018. During this study period no any microbes were isolated as a result of aerobic culture and no any fungal pathogen were isolated as a result of fungal culture (As shown in Table 2). Moisture contents main causative factor in drug deterioration, it also act as an enzymatic activator which slowly decompose the drug resulting in its degradation [10].

Conclusion

Shelf- life is 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. Hence Microbiological study of the *Amalakibhavita Nisha* showed the quality of *Vati* in standard condition. There were no growth of microorganisms (bacterial or fungal) found, till 30th April 2018 i.e. 15th month from the date of preparation of *Amalakibhavita Nisha*, which shows its good shelf life.

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