EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 7
215-222
Year: 2019

STABILITY STUDY OF *MADHUMEHARI VATI* USED IN THE MANAGEMENT OF TYPE 2 DIABETES *(MADHUMEHA)* - WITH RESPECT TO BASELINE MICROBIAL DIAGNOSTIC MODALITIES

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Article Received on 07/05/2019 **Article Revised on 27/05/2019** **Article Accepted on 17/06/2019**

ABSTARCT

Background: Diabetes mellitus (DM), is a group of metabolic disorders characterized by high blood sugar levels over a prolonged period. In *Ayurveda* disease Diabetes Mellitus can be correlated with *Madhumeha*. From different drugs described in *Ayurveda* for the management of *Madhumeha* (Type 2 Diabetes), a compound formulation has been made named *Madhumehari Vati*. In this formulation whole plant extract of *Mamajjak* (*Enicostemma littorale*), leaves extract of *Meshashringi* (*Gymnema sylvestre*), nut extract of *Latakaranja* (*Caesalpinia bonducella*), root extract of *Katuki* (*Picrorhiza kurroa*), fruit extract of *Pippali* (*Piper* longum), fruit extract of *Rakta-maricha* (*Capsicum frutescens*), fruit extract of *Indravaruni* (*Citrullus colocynthis*) were taken. **Aim**: To study the stability of *Madhumehari Vati* with respect to its microbial profile. **Materials and Methods:** Prepared sample of *Madhumehari Vati* was studied to check microbial contamination at regular time intervals. Tablets were studied at regular intervals of 1 month for a period of 09 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 to the date of last microbiological study. No any contaminations were found in microbiological study. **Discussion**: Hence the present Study was carried out to observe the stability study of *Madhumehari Vati* with respect to Microbial Contamination of sample prepared and store in different climatic conditions and temperature. Thus a baseline Microbial profile was studied at regular interval of 1 month for total 11 month (i.e. time for consumption of prepared drug). At the end of study it was found that sample was not showed presence of any Microbes. **Conclusion**: In microbiological study, Tablets container has not present of microbes after 11 months of preparation sample, even in different climate and temperature, shows its stability and good shelf life. Hence in present study the stability test of *Madhumehari Vati* with respect to microbiological findings was negative at room temperature, warm and cold, dry and humid conditions.

KEY WORDS: *Ayurveda***,** Climate conditions, Microbial profile, Stability, Type 2 Diabetes *(Madhumeha)*, *Madhumehari Vati*.

INTRODUCTION

Diabetes mellitus (DM), commonly known as diabetes, is a group of metabolic disorders characterized by high blood sugar levels over a prolonged period.[1] Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger.[2] If left untreated, diabetes can cause many complications.[3] Acute complications can include diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death.[4] Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, and damage to the eyes.[5] In *Ayurveda* disease type 2 diabetes can be correlated with *Madhumeha.* According to *Ayurveda* life style activities which increase *Kapha*, use of *Guru*

(heavy to digest), *Snigdha* (unctuous), *Atinidra* (excess sleep), *Avyayama* (lack of exercise), *Achinta* (lack of mental exercise) are the causes of *Madhumeha*.

For the first time the research work carried out for authentication and microbial profile of *Madhumehari Vati*. The drug was prepared and provided by Dr Vasishth's AyuRemedies (named as Glycie tablet) by adopting standard operative procedure for tablet formation. Drug preparation was finished on 01/02/2017.

Stability of a pharmaceutical product is the capability of a particular formulation in a specific container or closure system, to remain within its physical, chemical, microbiological therapeutic specifications. Thus in the present study on attempt was taken to check stability of tablet with respect to its Microbial profile at different climatic conditions and temperature setups at regular interval for a period of 09 months.

AIM: To study the stability of finished product and to check microbial contamination in the finished product at different time interval- at different climatic conditions, temperature and humidity set ups.

MATERIALS AND METHODS

Sample of *Madhumehari Vati* was prepared (stored at room temperature) and finished product studied to check microbial contamination at regular intervals of 1 month for a period of 09 months (up to 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 70^{h} 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 drug materials were identified and authenticated by the Pharmacognosy department, IPGT & RA, GAU, Jamnagar. The ingredients and the part used are given in table 1.

Table-1: Ingredients of *Madhumehari Vati* (*Anubhut Yoga*)

Drug	**Botanical name**	**Parts used** (Dry)	**Quantity**
Mamajjak	*Enicostemma littorale* Blume	*Panchanga*	300mg
Meshashringi	*Gymnema sylvestre* R.Br	Leaves	250mg
Latakaranja	*Caesalpinia bonducella* (Linn.) Roxb.	Nut	150mg
Katuki	*Picrorhiza kurroa* Royle ex Benth.	Root	50mg
Pippali	*Piper longam* Linn.	Fruit	40mg
Rakta maricha	*Capsicum frutescens* Linn.	Fruit	8mg
Indravaruni	*Citrullus colocynthis* Linn.	Fruit	2mg

Date of Drug Preparation: 1st February 2017

Specimen: *Madhumehari Vati*

Storage

Finished product of *Madhumehari Vati* was stored in air-tight food grade, plastic containers, stored in the open light area in the department at room temperature.

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

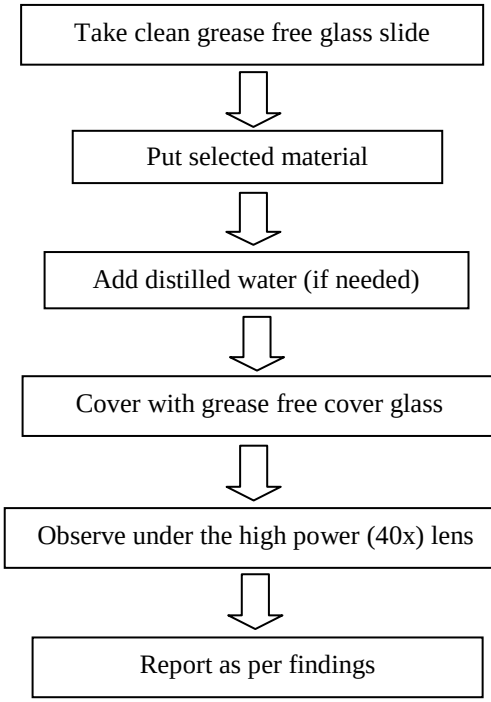
The details of the procedures followed are given below:

1. Smear Examination:

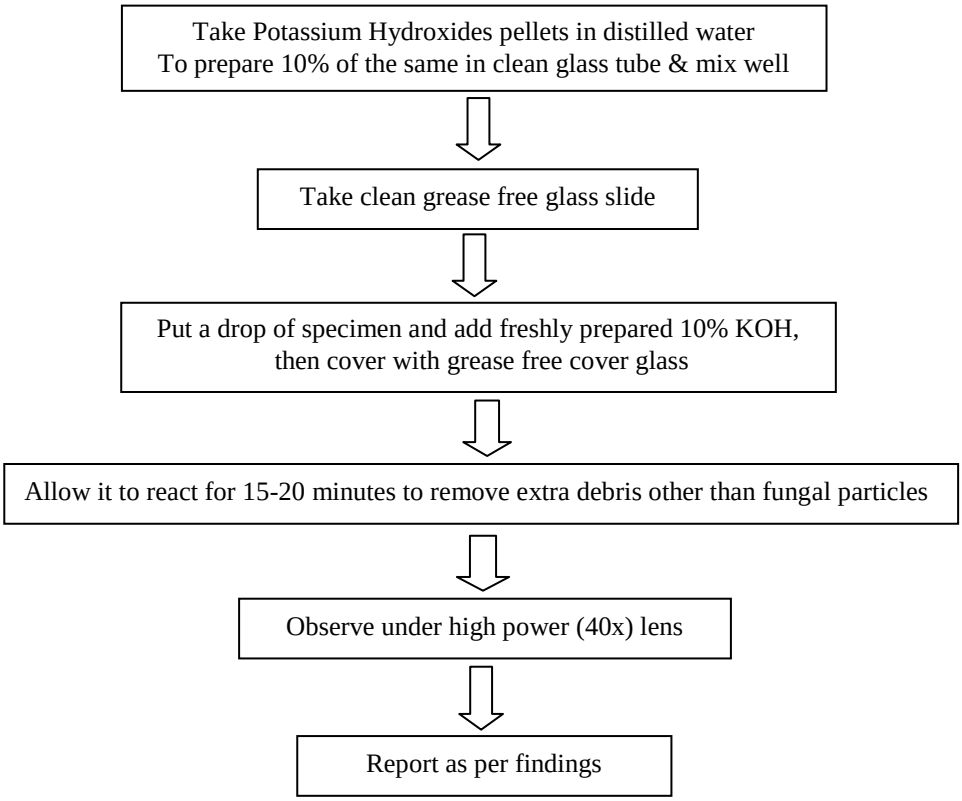
A. Wet mount /10% K.O.H. Preparation

Aim: To rule out any mycological findings.

Procedure for Wet Preparation



Procedure For 10% KOH Preparation



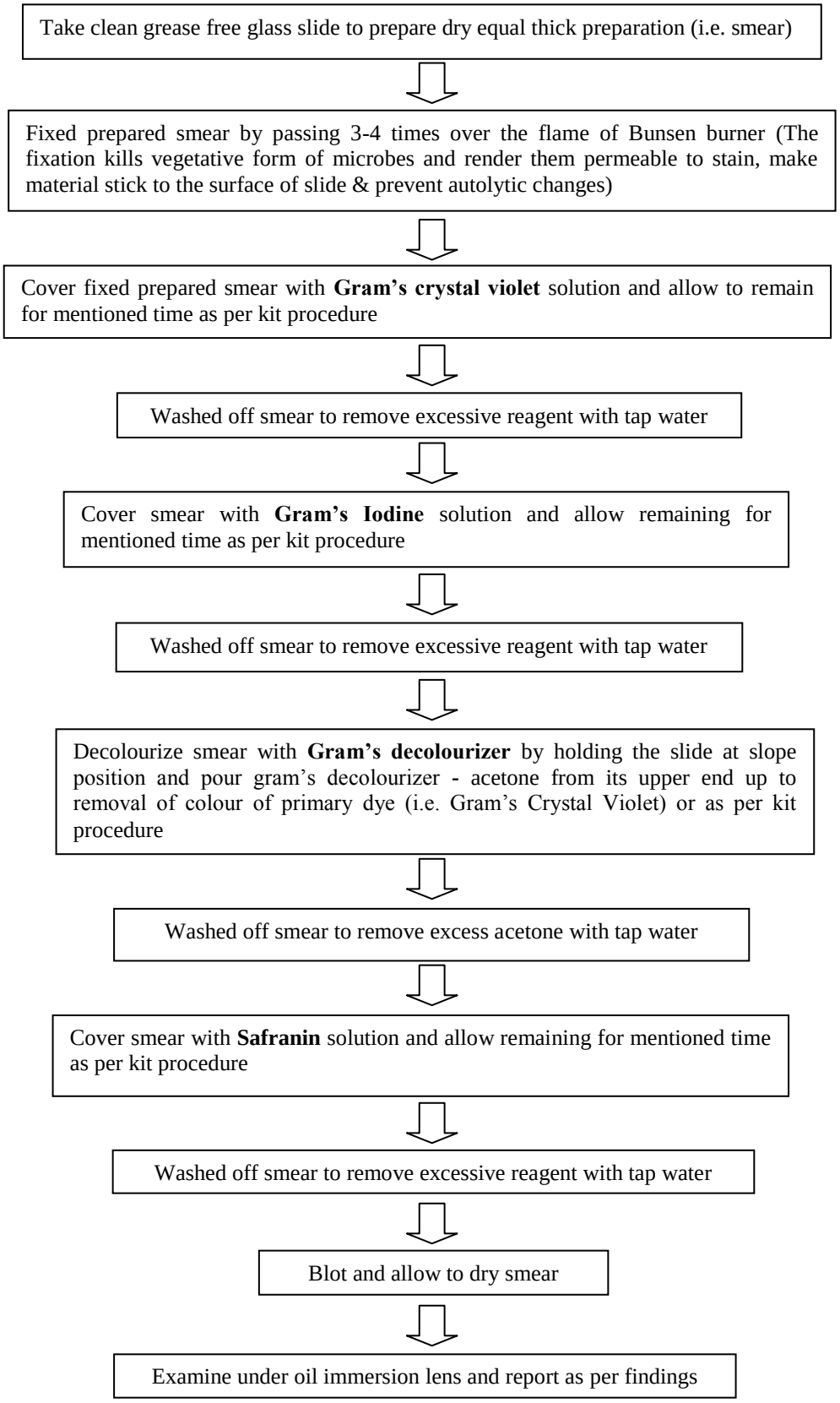
B. Gram's stain test

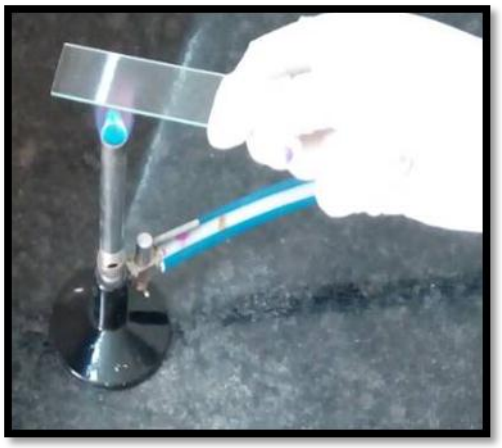
Gram staining is a differential staining technique that differentiates bacteria into two groups: gram positive and gram negative. The procedure is based on the ability of microorganisms to retain colour of the stains used during the gram stain procedure. Gram negative bacteria are decolorized by any 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 decolorization step, a counter stain effect 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 (Alfred E Brown, 2001).[6]

Aim: To rule out any bacteriological findings.
Specimen: *Madhumehari Vati.*

Procedure for Gram's Stain





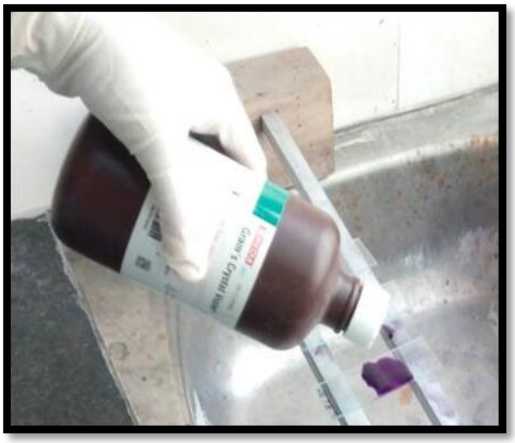


Figure 1 & 2: Smear staining Procedure.

Figure 3: Stained smear ready for examination

2. CULTURE STUDY

A. 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 : 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 ºC
Use of media : For selective cultivation of pathogenic fungi.

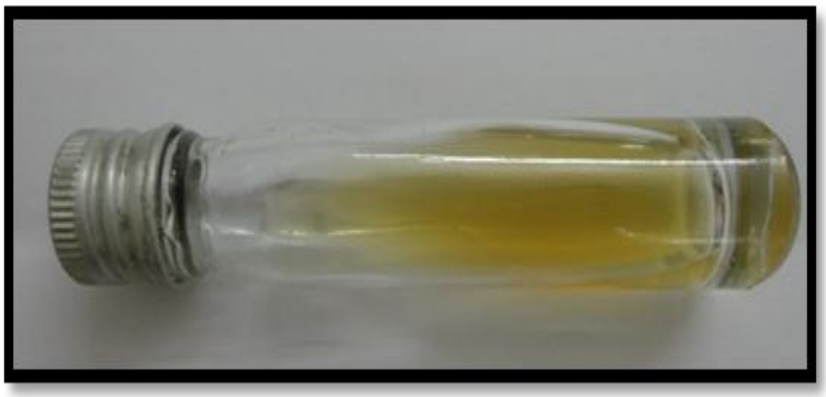


Figure 4 - Sabouraud Dextrose Agar Base (SDA)

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

⇩

Dry selective solid media in Hot Air Oven **before** specimen inoculation
Allow to cool 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 cool than loop is charged with 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 37^0 C for 5 to 7 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 aerial growth and confirm growth by performing different related biochemical reactions and different related staining procedures. After that report isolates.

B. Aerobic culture method

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.

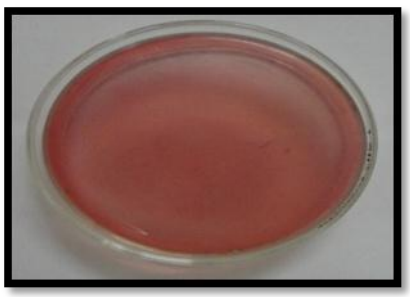


Figure 5: Aerobic culture media (MA)

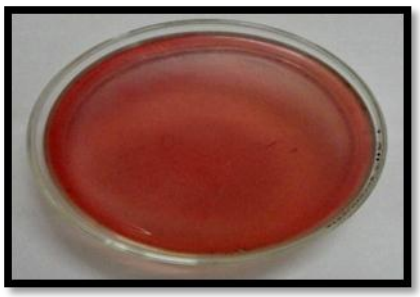


Figure 6: Aerobic culture media (BA)

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 37^0c 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

OBSERVATIONS AND 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 study	Temp.	Humidity	Observations of Both samples			
				Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
1.	2nd month (13/04/2017)	41°C	30%	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
2.	3th month (17/05/2017)	43°C	35%	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
3.	4th month (13/06/2017)	41°C	38%	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
4.	5th month (18/07/2017)	32°C	74%	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
5.	6th month (23/08/2017)	30°C	80%	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
6.	7th month (21/09/2017)	33°C	69%	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
7.	8th month (12/10/2017)	33°C	61%	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
8.	9th month (16/11/2017)	34°C	30%	Microorganisms not seen	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
9.	11th month (17/01/2018)	29°C	35	Microorganisms 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). *Madhumehari Vati* is never used before for the research work at IPGT & RA and elsewhere in India. In present study, it has shown a very good and promising result in management of *Madhumeha* (Type 2 Diabetes). Hence the present Study was carried out to observe the stability study of *Madhumehari Vati* with respect to Microbial Contamination of sample prepared and preserved in different climatic and temperature conditions. Thus a baseline Microbial profile was studied at regular interval of 1 month for total 09 months. At the end of study it was found that sample has not shown presence of any Microbes.

The term drug stability refers to the extent to which a drug substance or product retains, within specified limits and throughout its period of storage and use, the same properties and characteristics that it possessed at the time of its manufacture. The type of stability is generally divided into chemical, physical, microbiological, therapeutic, and toxicological.[7] Drug stability can be categorized as pre-market and commercial (marketed product) stability. Pre-market stability, which supports the clinical trial where drug products are stored under different conditions for safety and efficacy evaluation, is usually conducted throughout the clinical trial and during the filing period. Commercial stability is continuous assurance on the post-approval batches for long-term stability monitoring on the drug product. Drug stability assessment generally involves the testing of the drug substance or drug product using a stability-indicating method in order to establish the retest period (for pre-market stability) and shelf life (for commercial stability).[8]

Madhumehari Vati was prepared and stored at room temperature. Sample was selected randomly for study of microbiological contamination. Changes in temperature and humidity of environment were observed during study period. High temperature leads to drug degradation by accelerating oxidation, reduction and hydrolysis. Moisture contents main causative factor in drug deterioration, it also act as an enzymatic activator which slowly decompose the drug resulting in its degradation.[9] Water catalyze chemical reaction by oxidation, hydrolysis, reduction and promotes microbial growths. 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. High RH may allow the growth of microbes,[10] 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 17th January 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).

CONCLUSION

Stability testing is an important part of the drug development and approval process, determining the safety and integrity of the drug and also its shelf life and storage conditions. 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. Sources of Microbial Contamination are Water, Air, Raw materials, containers and closures, Personnel, Instruments and apparatus. Hence Microbiological study of the *Madhumehari Vati* showed that the quality of Tablet in standard condition. There were no growth of microorganisms (bacterial or fungal) found, till 17th January 2018 i.e. 11th month from the date of preparation of *Madhumehari Vati*, which shows their good shelf life.

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