Original Research Paper

Volume-9 | Issue-2 | February-2019 | PRINT ISSN - 2249-555X

Ayurveda

MICROBIOLOGICAL STUDY OF SHWASAHARA DASHEMANI WITH RESPECT TO BASELINE MICROBIAL PROFILE USED IN *TAMAKA SHWASA*

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ABSTRACT *Tamaka Shwasa* is basically a disorder of *Praanavaha Srootasas* while other *Srootasas* are also vitiated. The parallel disease entity in contemporary medical science to this disorder is Bronchial Asthma. Bronchial Asthma is a chronic inflammatory condition of the lung airways resulting in episodic airflow obstruction. This disease is more predominant in children and aged population. In *Charaka Samhita*, the group of ten drugs is mentioned for the management of the *Shwasa Roga* named as *Shwasahara Dashemani*. Palatability is a main issue in treatment of children so keep it mind the *Avaleha* (Group A) and *Churna* (Group B) form is prepared which is very easily palatable in children. In present study, stability with respect to its Microbial profile of *Shwasahara Dashemani* was carried out. *Avaleha* (Group A) and *churna* (Group B) were stored in plastic bags with in container during different climacteric conditions were studied at particular time intervals for a period of 393 days (Group A) and 401 days (Group B) to analysis Mycological findings and presence of bacteriological findings by Wet mount preparation and Gram stain test respectively. At the end of study any *Shwasahara Dashemani* bag has not present with any microbes after 393(Group A) and 401 days(Group B) of preparation sample, even in different climate and temperature. Hence in present study the stability test of *Shwasahara Dashemani* with respect to microbiological findings was negative at room temperature, warm and cold, dry and humid conditions.

KEYWORDS : Stability, Microbial profile, *Shwasahara Dashemani*

INTRODUCTION:

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 and toxicological specifications at a defined storage condition. Thus the present study was designed to study the stability of *Swasahara Dashemani Avaleha and Swasahara Dashemani Churna* with respect to microbial contamination after a period of about 393 days and 401 days of preparation in group A (*Swasahara Dashemani Avaleha*) and group B (*Swasahara Dashemani churna*) respectively. The study was conducted at 12 different time interval at different climatic conditions and temperature set ups.

AIM:

To study the microbial contamination in the finished product at different time interval.

MATERIALS AND METHODS:

Samples of *Shwasahara Dashemani Avleha* Group A, And Group B *Shwasahara Dashemani Churna* were prepared and studied to check microbial contamination at different climatic conditions. The study was conducted at Microbiology Laboratory, IPGT & RA, Jamnagar, India.

Preparation Time:

Both the drugs were prepared separately with the utmost care to avoid any sort of contamination.

Date of preparation:

- **Group A:** 03/1/2017;
- **Group B:** 26/12/2016

STORAGE:

Drugs of Group A was stored in plastic air tight containers and Group B was stored in Plastic bags at room temperature in a dark and dry place. Both the samples were subjected to stability study with respect to microbial and fungal contamination at regular intervals. Details of which are cited below.

Microbial profile: Microbial contamination was assessed by two method stocheckany mycological findings and bacteriological findings.

A. Wet mount /10% K.O.H. Preparation and Fungal culture.
B. Gram's stain test and aerobic culture media.

The details of the procedures followed are given below.

A. Wet mount /10% K.O.H. Preparation and Fungal culture:

Aim: To rule out any mycological findings.

Specimen:

1. Group A (*Shwasahara Dashemani Avaleha* prepared same Sample at 12 different time interval.
2. Group B (*Shwasahara Dashemani Churna* prepared with one *Bhavana*) same Sample at 12 different time interval.

Procedure:

1.Smear preparation and examination:

Both the selected samples (Group A and Group B) were taken on grease free glass slides + 10% KOH and covered with clean cover slips for microscopic examination.

2.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

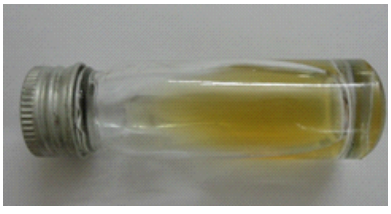

FIGURE : FUNGAL CULTURE MEDIA

B. Gram's stain test and aerobic culture media:

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 color 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)[ii]

Aim: To rule out any bacteriological findings.

Specimen:

1. Group A (*Shwasahara Dashemani Avaleha* same Sample at 12 different time interval.
2. Group B (*Shwasahara Dashemani Churna* prepared with one *Bhavana*) same Sample at 12 different time interval.

1. Smear preparation and examination:

Selected samples (Group A and Group B) were taken and smear was prepared on grease free glass slides, and stained as per staining procedure mentioned below. After drying of smear, examination was done in oil immersion lens to render any bacteriological findings.

PROCEDURE:

The smear was covered with crystal violet and allowed to remain for mentioned time as per kit procedure. Then the stain was washed off, using a wash bottle of distilled water/tap water. Excess water was drained off.

In second step the smear was covered with Gram's iodine solution and allowed to remain for mentioned time as per kit procedure. Gram's iodine was later poured off and the smear was washed off, using a wash bottle of distilled water/tap water.

In third step the smear was flooded with Gram's decolorizer i.e. acetone for mentioned time as per kit procedure. The excess acetone was removed by rinsing the slide with distilled water/tap water.

In fourth step the smear was covered with saffron in for mentioned time as per kit procedure followed by distilled water/tap water wash and allowed to air dry. The slide was examined under oil immersion.

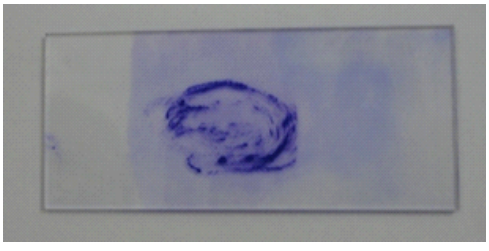

Figure: Stained smear ready for examination

2. 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 : Mac Conkey Agar (MA) and Coulmbia 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.

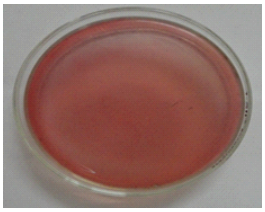

Figure: Aerobic culture media (MA)

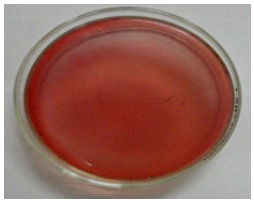

Figure: Aerobic culture media (BA)

OBSERVATIONS:

Following findings were observed at the end of the study. Table show the observations of group A and group B respectively during the tests at regular intervals after 393 days in group A and 401 days in group B from the date of preparation of the drug.

OBSERVATION OF GROUP A:

Samples of group A preserved at room temperature did not show the presence of any mycological or bacteriological findings at the end of 12 different time interval observation after 265 days after the preparation of drug. The study was done after 43 days, 73 days, 106 days, 136 days and 171 days, 202 days, 238 days, 265 days, 290 days, 318days, 352 days, & 393 days from the day of preparation respectively.

OBSERVATION OF GROUP B:

Samples of group B preserved at room temperature did not show the presence of any mycological or bacteriological findings at the end of 12 different time interval observation after 401 days after the preparation of drug. The study was done after 51 days, 81 days, 114 days, 144 days and 179 days, 210 days, 246 days, 273 days, 298days, 326 days, 360 days and 401 days from the day of preparation respectively.

All the samples of group A and B at different time interval showed negative finding of bacterial as well as mycological contamination. The actual temperature of Jamnagar city at the time of study is mentioned in the tables 3 and 4 for group A and group B respectively.[iii]

Table : Observations Of Sample A Preserved At Room Temperature.

Sr. No.	Days of investigations After preparation of the sample	Storage temperature/ Humidity		Observations of sample A			
		Humidity	Temp. (^{0}C)	Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
1.	43 days	17%	(35^0-19^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
2.	73 Days	20%	(30^0-16^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
3.	106 Days	73%	(40^0-24^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
4.	136 Days	34%	(42^0-24^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
5.	171 Days	75%	(45^0-28^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
6.	202 days	89%	(39^0-25^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated

7.	238 days	90%	(40^0-23^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
8.	265 days	59%	(37^0-24^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
9	290 days	26%	(38^0-24^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
10	318 days	28%	(34^0-22^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
11	352 days	28%	(28^0-21^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
12	393 days	25%	(34^0-19^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated

Table : Observations of sample B preserved at room temperature.

Sr. No.	Days of investigations After preparation of the sample	Storage temperature/ Humidity		Observations of sample B			
		Humidity	Temp. (^{0}C)	Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
1.	51 Days	17%	(35^0-19^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
2.	81 Days	20%	(30^0-16^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
3.	114 Days	73%	(40^0-24^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
4.	144 Days	34%	(42^0-24^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
5.	179 Days	75%	(45^0-28^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
6.	210 Days	89%	(39^0-25^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
7.	246 Days	90%	(40^0-23^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
8.	273 days	59%	(37^0-24^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
9	298 days	26%	(38^0-24^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
10	326 days	28%	(34^0-22^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
11	360 days	28%	(28^0-21^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
12	401 days	25%	(34^0-19^0)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated

DISCUSSION:

Bronchial asthma is a burning health issue in worldwide. It is a very common disease in young generation because easily inflamed by infected environment. Bronchial Asthma is a common cause of school absentism in young child. Till date no effected medicine is discovered to cure the bronchial Asthma without any side effect. Ayurveda provide a effected medicine named as *Shwasahara Dashemani* i.e. to improve the health status and quality of life in *Tamaka Shwasa* (Bronchial Asthma). Stability study of *Shwasahara Dashemani* with respect to Microbial Contamination of sample prepared and preserved in different climacteric and temperature conditions. Thus a baseline Microbial profile was studied at particular interval of days. At the end of study it was observed all the containers have not shown presence of any Microbes.

Stability is usually expressed in term of self-life, which is the time period from when the product is produced until the time it is intended to be consumed or used. Microorganisms need water, Humidity, Temperature at suitable environmental conditions to develop in any media, surface and article.

CONCLUSION:

Self 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 self-life, ranging from organoleptic qualities to microbiological safety. Hence Microbiological study of the *Shwasahara Dashemani* showed that the quality of *Avaleha* and *Churna* in a standard condition. There were no growth found of microorganisms (Bacterial or fungal) till March 2018 i.e. More than 1 year from the date of preparation, shows its good shelf life.

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