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STABILITY STUDY OF PATOLADI KWATH FOR THE ASSESSMENT OF BASELINE MICROBIAL PROFILE USED IN MIGRAINE ASSOCIATED GASTROINTESTINAL DISEASES W.S.R. TO AMLAPITTA

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ABSTRACT

Migraine is the most prevalent neurological disorder which is also associated with gastrointestinal symptoms. *Patoladi Kwath* is one of the herbal formulations mentioned which was used in clinical trial to treat Migraine associated with *Amlapitta*. **Objective:** In the present study, stability with respect to its microbial profile in different climate condition of *Patoladi Kwath* carried out. **Methods:** *Patoladi Kwath* was stored in plastic bag of 1 kg each with numbers given to each bag in cool, dark and dry place during different climatic condition. The drug was studied at different intervals for a period of one and half year from March 2019 to October 2020 for the assessment of mycological findings and presence of microorganisms by Wet mount preparation and Gram stain test respectively. **Results and Conclusions:** At the end of study, no contamination found in prepared drug at minimum humidity of 52% with 36^0C temperature and at maximum humidity of 94% with 27^0C temperature. Contamination in form of the bacteria and fungus at minimum humidity of 42% with 32^0C temperature and at maximum humidity of 91% with 29^0C temperature found in prepared drug.

Key words: Microbial profile, *Patoladi Kwath*, Stability study, Climate condition.

INTRODUCTION

Migraine is the most prevalent neurological disorder reported in Global Burden of Diseases by WHO. In India, 488 million people were affected with Migraine in 2019. As per *Ayurveda*, this disease can be correlated with *Ardhavabhedaka* due to similarity of its causes, symptoms and paroxysmal nature of the disease. The previous research studies are found with data that migraineurs are mostly having previous history of *Amlapitta* (~Hyperacidity i.e., heartburn, acid reflux, nausea, vomiting etc.). So, for the clinical study, *Patoladi Kwath* was selected and for the stability of the finished drug the microbial profile was checked.

Yavakuta (coarse powder) of all the four ingredients (each 25 kg) of *Patoladi Kwath* was made, in Pharmacy, Guajarat Ayurved University, Jamnagar, under standard operating procedure and with proper precautions to avoid any contamination. The preparation of the drug was finished on 08-03-2019. Then, the prepared drug was packed in plastic bag of 1 kg each and given numbers to them. These bags are kept in cool, dark and dry place in the department. This finished drug was given to the patients of migraine associated with *Amlapitta*. This formulation was first checked and assured with nil microbial contamination prior to give it to the patients. For that, this study has been planned to check stability of finished drug to its microbial profile at different climacteric conditions and temperature with regular interval of the time. The stability study was performed approximately one and half year.

AIM:

To study the microbial contamination in *Patoladi Kwath* (coarse powder) at different time interval at different conditions of weather i.e., temperature, humidity etc.

Materials and Methods:

Sample of *Patoladi Kwath* was prepared (stored at room temperature) and studied to check microbial contamination at regular intervals for a period of one and half year. Microbiological study has been carried out in Microbiology Laboratory, IPGT & RA, GAU, Jamnagar, Gujarat. Mainly two studies have been carried out to rule out that presence of any bacteria or fungi in the test drug.

The initial microbiological study was done before giving it to the patients. Then samples from plastic bags were collected from plastic bags for the microbiological study regularly with random intervals during different seasons with different climates and temperatures.

Contents of samples:

The sample contents approximately 0.25 gm of *Patoladi Kwath* coarse powder which includes four ingredients i.e., Trichosanthes dioca Roxb., Coriandrum sativum Linn., Zingiber officinale Roscoe., Cyperus rotundus Linn. [1] All the raw drugs were procured from the raw drug store of pharmacy, GAU, Jamnagar, Gujarat.

Preparation of the drug:

The drug was prepared in Gujarat Ayurved University Pharmacy with the standard operating procedure and utmost care for avoiding contamination.

Date of preparation: 08.03.2019

Storage:

Finished product, coarse powder of *Patoladi Kwath* was stored in plastic bags of 1 kg each at room temperature in a dark and dry place. So, the bag no. was assigned for testing. Samples were subjected to stability study for microbial and fungal contamination at different intervals of time with proper precautions for avoiding contamination. Details of which are cited below.

Microbial profile: Two methods were implied for microbial contamination and to check any mycological findings and bacteriological findings.

1. **Smear Examination-**

A) Gram's stain

B) 10% K.O.H. Preparation

2. **Culture Study-**

A) Aerobic culture

B) Fungal culture

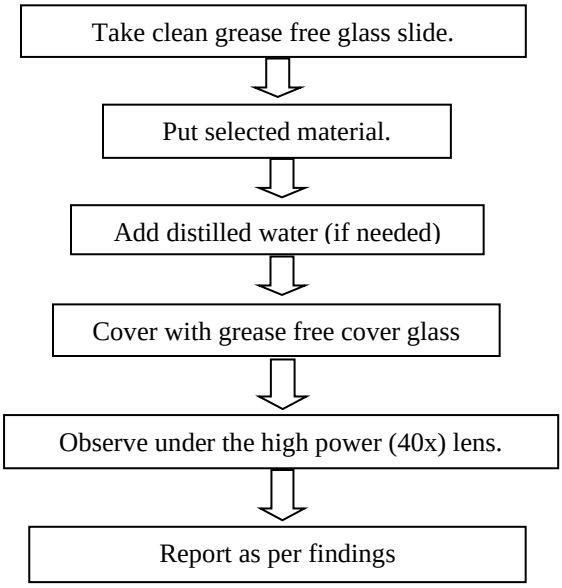
The details of the procedures followed are given below.

1. Smear Examination:

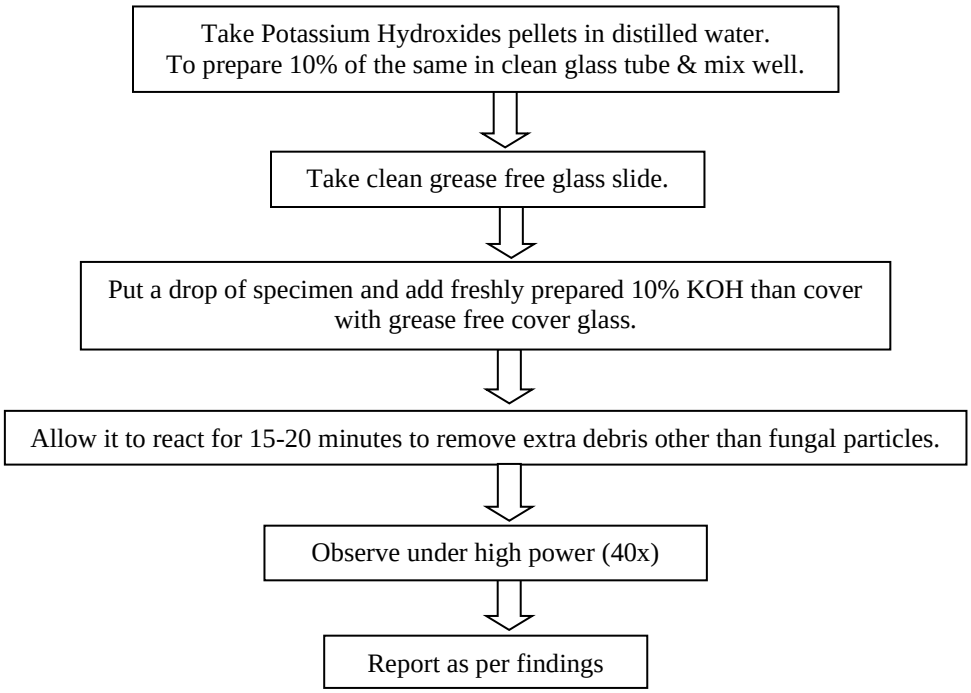
A. 10% K.O.H. preparation: [2]

Aim: To rule out any mycological findings. **Specimen:** ***Patoladi Kwath***

- **Procedure for wet mount preparation**



- **Procedure For 10% KOH Preparation**



B. Gram's stain test: [3]

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 the 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 purple. After the decolorization step, a counterstain effect found on gram-negative bacteria and bacteria will remain pink. The Gram stain procedure enables bacteria to retain the color of the stains, based on the differences in the chemical and physical properties of the cell wall.

Aim: To rule out any bacteriological findings.

Specimen: ***Patoladi Kwath***

- **Procedure For Gram's Stain:**

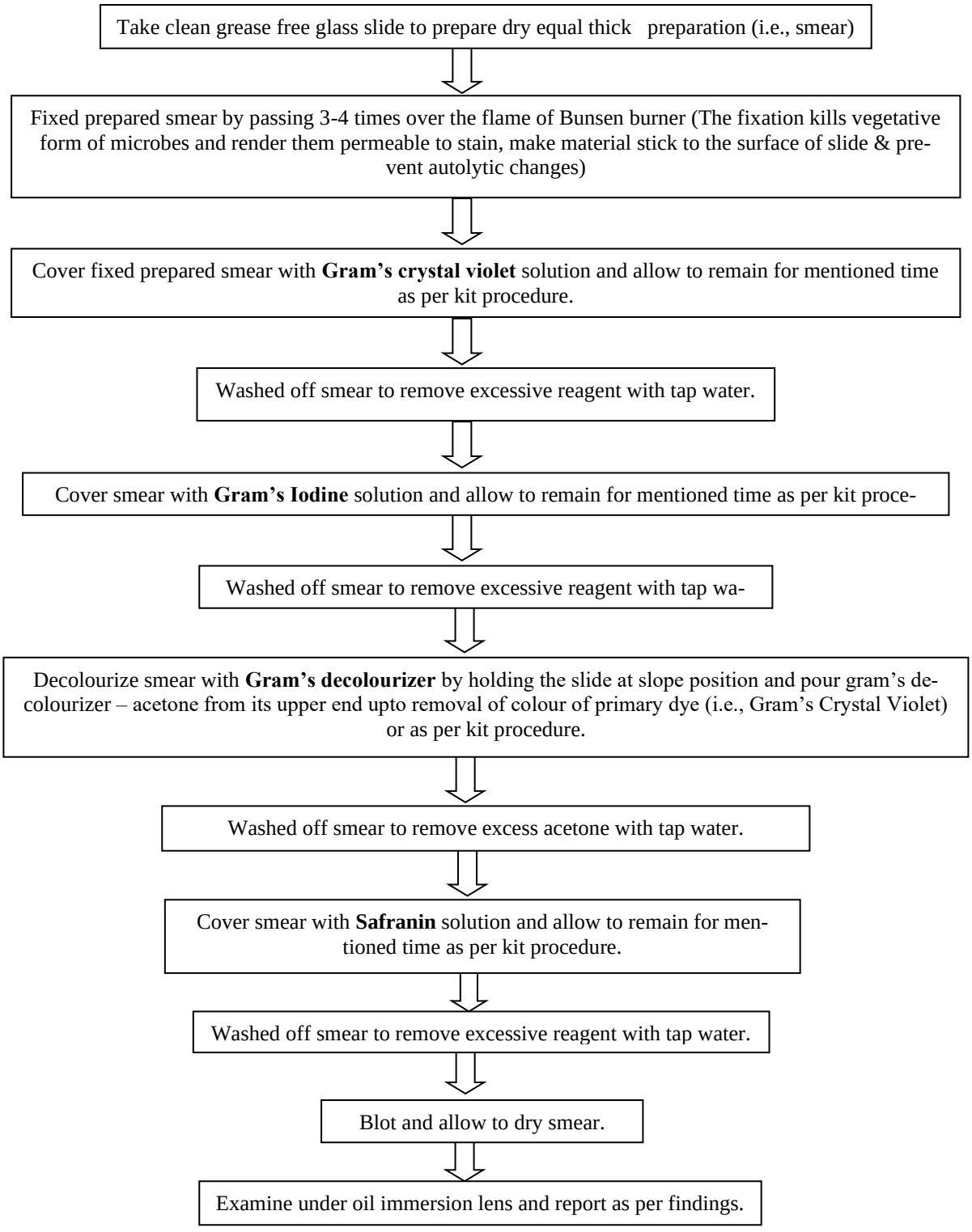


Figure 1, 2 - Smear staining Procedure

- **Culture study:**

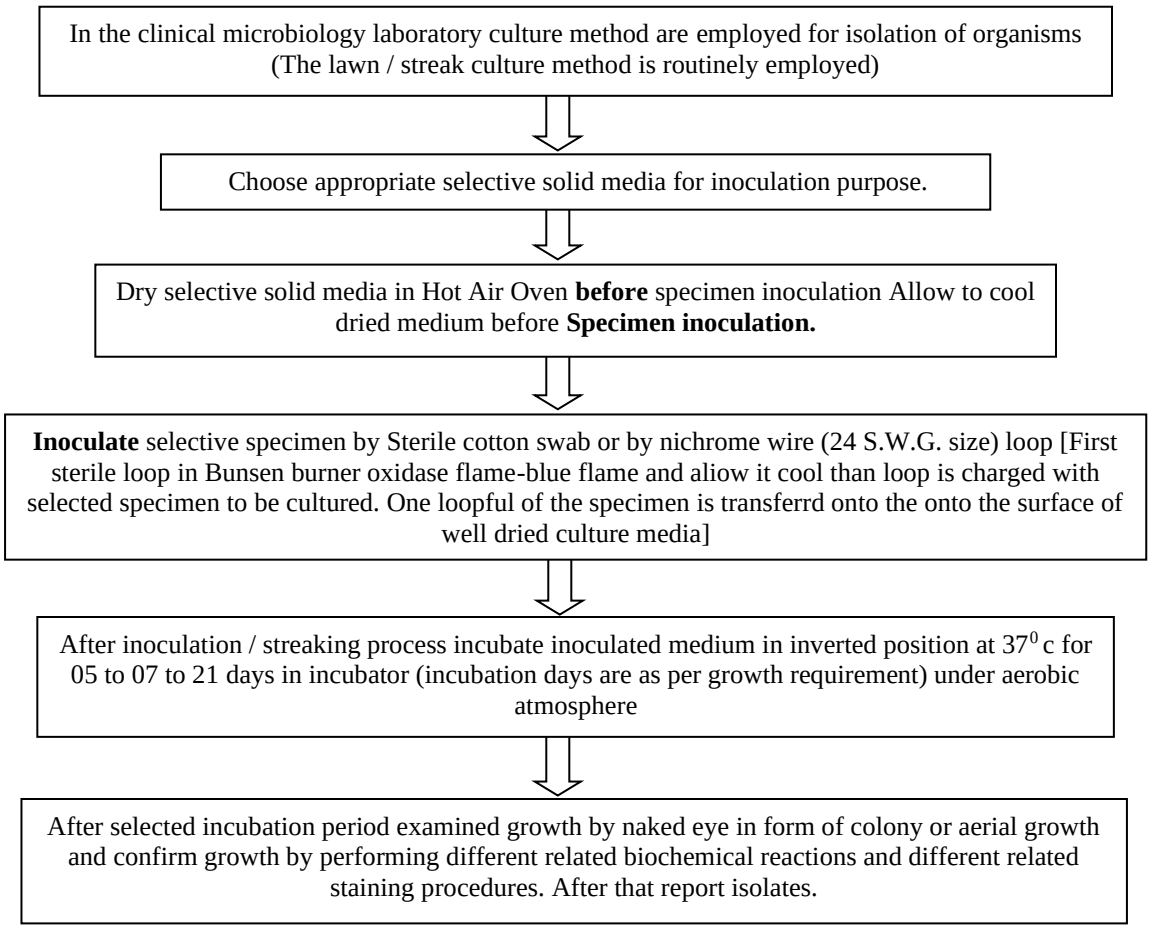
A. Fungal culture method: [4]

Respected materials were collected with a sterile cotton swab for inoculation purposes 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 3: Sabouraud Dextrose Agar Base (SDA) bottle

- **Procedure For Fungal Culture**

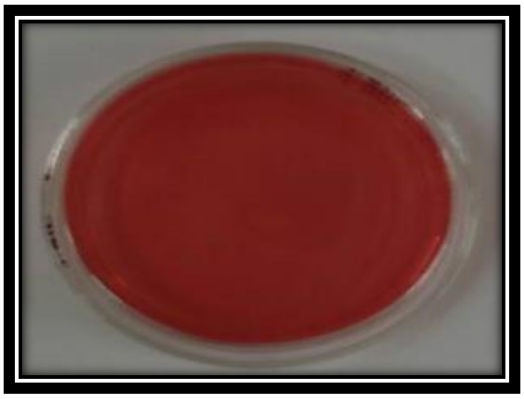


B. Aerobic culture method: [5]

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 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 4. Mac Conkey Agar (MA)



- **<u>Procedure For Aerobic Culture</u>**

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 sreaks 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 Result:

Table-1: Showing observations of a sample of *Patoladi Kwath* preserved at room temperature.

Sr. No	Days of investigation after preparation of the sample at	Bag No. & Date of Sample given	Humidity and Temp.	Observations of sample			
				Gram's Stain	Aerobic culture report	10% K.O.H. Preparation	Fungal culture report
1.	28 Days	Bag No. 1 03/04/2019	80% 30^0C	Many gram-negative rods were seen	Pseudomonas aeruginosa	Fungal filaments not seen.	No fungal pathogen isolated
2.	37 Days	Bag No. 6 12/04/2019	80% 30^0C	Many gram-negative rods were seen	Pseudomonas aeruginosa	Fungal filaments not seen.	No fungal pathogen isolated
3.	43 Days	Bag No. 10 18/04/2019	72% 29^0C	Many gram-negative rods were seen	Pseudomonas aeruginosa	Fungal filaments not seen.	No fungal pathogen isolated
4.	54 Days	Bag No. 11-20 29/04/2019	73% 30^0C	Absence of micro-organisms	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
5.	103 Days	Bag No. 24 17/06/2019	86% 31^0C	Absence of micro-organisms	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
6.	173 Days	Bag No. 40-54 26/08/2019	84% 28^0C	Absence of micro-organisms	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
7.	173 Days	Bag No. 55-62 26/08/2019	84% 28^0C	Absence of micro-organisms	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
8.	195 Days	Bag No. 63-64 17/09/2019	90% 30^0C	Absence of micro-organisms	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
9.	211 Days	Bag No. 65-69 03/10/2019	82% 31^0C	Absence of micro-organisms	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
10.	230 Days	Bag No. 70-75 22/10/2019	78% 28^0C	Absence of micro-organisms	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
11.	250 Days	Bag No. 78-79 11/11/2019	42% 32^0C	Many gram-negative rods were seen	Pseudomonas aeruginosa	Fungal filaments are seen.	Aspergillus flavus seen
12.	259 Days	Bag No. 80-82 20/11/2019	52% 31^0C	Many gram-negative rods were seen	Pseudomonas aeruginosa	Fungal filaments are seen.	Aspergillus flavus seen
13.	274 Days	Bag No. 83-84 05/12/2019	68% 29^0C	Absence of micro-organisms	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
14.	275 Days	Bag No. 85 06/12/2019	61% 29^0C	Absence of micro-organisms	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
15.	295 Days	Bag No. 86-87 26/12/2019	67% 23^0C	Absence of micro-organisms	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
16.	302 Days	Bag No. 88-89 02/01/2020	80% 23^0C	Absence of micro-	No organisms isolated	Fungal filaments not seen.	No fungal pathogen

				organisms			isolated
17.	323 Days	Bag No. 90-91 23/01/2020	71% 27^{0}C	Absence of micro-organisms	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
18.	476 Days	Bag No. 92-93 24/06/2020	84% 32^{0}C	Absence of micro-organisms	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
19.	482 Days	Bag No. 94-95 30/06/2020	94% 27^{0}C	Absence of micro-organisms	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated
20.	505 Days	Bag No. 96 -97 23/07/2020	82% 29^{0}C	Many pleomorphic gram-negative rods were seen	Pseudomonas aeruginosa & Escherichia coli found	Fungal filaments not seen.	No fungal pathogen isolated
21.	512 Days	Bag No. 94-95 30/07/2020	85% 30^{0}C	Many capsulated gram-negative rods were seen	Escherichia coli found	Fungal filaments are seen.	Aspergillus flavus seen
22.	519 Days	Bag No. 96-99 06/08/2020	91% 29^{0}C	Many capsulated gram-negative rods were seen	Escherichia coli found	Fungal filaments are seen.	Aspergillus flavus seen
23.	523 Days	Bag No. 95-99 10/08/2020	88% 30^{0}C	Many capsulated gram-negative rods were seen	Escherichia coli found	Fungal filaments are seen.	Aspergillus flavus seen
24.	16 Days	New Lot 14/10/2020	52% 36^{0}C	Absence of micro-organisms	No organisms isolated	Fungal filaments not seen.	No fungal pathogen isolated

DISCUSSION

Ayurveda, a science of life, gives promising results in many diseases like Migraine in which no promising results are established. In this research study, *Patoladi Kwath* has been chose to give the patients enrolled in the clinical study for the management of Migraine associated with *Amlapitta*. For the safety purpose, it is needed to be proved safe on microbiological profile. Hence the present study was carried out to observe the stability study of *Patoladi Kwath* with respect to microbial contamination of sample prepared and preserved in different climacteric and temperature conditions. The area where the medicine was manufactured, and the sample was kept as close to the seaside; also boasts the most extended seashore and the most seaports. Therefore, relative humidity (RH) is consistently high throughout the year, regardless of the season. The highest RH recorded was 94% in June, while the lowest RH was recorded in November at 42%. High RH might facilitate microorganism growth.[6] For long term storage, moisture content of drug plays a key role in seacoast area. Moisture contents also acts as an enzymatic activator which slowly decompose the drug resulting in its degradations well as drug deterioration.[7]

Thus, a baseline Microbial profile was studied at interval of 1 month approximately for about one and half year. At the end of study, it was observed that microbial contamination in the prepared drug at some temperature and humidity of the atmosphere as per shown in the table of observation.

CONCLUSION

At different time interval, prepared drug named *Patoladi Kwath* checked at Microbiology laboratory, IPGT & RA to rule out microbial contamination in prepared form (coarse powder) of final product. 42% minimum humidity and 94% maximum humidity and temperature range from 23^{0}C to 36^{0}C was found dur-

ing total study period from March 2019 to October 2020. Contamination found in form of the bacteria and fungus at various humidity of 42%, 52%, 72%, 80%, 82%, 85%, 88%, 91% and at various temperature of 32^0C, 31^0C, 29^0C, 30^0C, 29^0C, 30^0C, 30^0C, 29^0C respectively. No contamination found at humidity of 73%, 86%, 84%, 90%, 82%, 78%, 68%, 61%, 67%, 80%, 71%, 84%, 94%, 52% at various temperature of 30^0C, 31^0C, 28^0C, 30^0C, 31^0C, 28^0C, 29^0C, 29^0C, 23^0C, 23^0C, 27^0C, 32^0C, 27^0C, 36^0C respectively.

Stability of prepared drug found at minimum humidity of 52% with 36^0C temperature and at maximum humidity of 94% with 27^0C temperature. Contamination of prepared drug in form of the bacteria and fungus at minimum humidity of 42% with 32^0C temperature and at maximum humidity of 91% with 29^0C temperature.

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