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Research Article

A BASELINE MICROBIAL STABILITY STUDY OF *PANCHAVALKALADI TAILA* AND *DASHAMOOLA TAILA*- AN AYURVEDIC FORMULATION FOR PELVIC INFLAMMATORY DISEASE

Matangee Pandya[1]*, Meera Cholera[2], Shilpa Donga[3]
*[1]Ph.D Scholar, Department of Prasutitantra Evum Streeroga, [2]Head of Microbiology Laboratory, [3]Professor, Department of Prasutitantra Evum Streeroga, Institute of Teaching and Research in Ayurveda, Jamnagar, India.

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ABSTRACT

Pelvic inflammatory disease is an upper genital tract infection and inflammation. *Panchavalkaladi Taila* and *Dashamoola Taila* is having anti-inflammatory and analgesic property. **Aim and Objectives**: Present study was carried out for stability study of *Panchavalkaladi Taila* and *Dashamoola Taila* with respect to microbial diagnostic procedure. **Materials and Methods**: Sample of *Panchavalkaladi Taila* and *Dashamoola Taila* were prepared and studied at regular time intervals from the date of the preparation to the date of completion of clinic study to analysis mycological findings by Wet mount preparation and presence of bacteriological findings by Gram stain test. **Result:** Both samples were subjected to the microbiological study from date of preparation to the date of completion of clinical study. No any contaminations were found in microbiological study in both samples. **Discussion and Conclusion**: The baseline microbial profile was studied at regular interval of one month for total 15 month (between October 2020 to January 2022). At the end of every month study, samples were not showed presence of any microbes even in different temperature and humidity of this sea sore region. **Conclusion:** Obtained data of *Panchavalkaladi Taila* and *Dashamoola Taila* were concluded that both samples were showed good shelf life.

INTRODUCTION

Multi-microbial bacteria move in ascending direction from cervix to uterus, ovaries and fallopian tubes that creates Pelvic Inflammatory Disease (PID). The bacteria can lead to an abscess in a fallopian tube or ovary. Long-term problems can occur if PID is not treated promptly.[1] Anti-inflammatory and anti-bacterial drugs can be used in this condition. Identified phytochemical components of *Panchavalkaladi* and *Dashamoola Taila* like tannins, anthraquinones, steroids, alkaloids, phytosterols all are astringents and anti-inflammatory which reduce the discharge, pain, tenderness, redness and swelling leading to quicker epithelization.

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Humidity, temperature, storage condition and microbial contamination factors affect the shelf life of drugs. Stability study depends on these factors and prove the shelf life of drug. In also Ayurvedic literature, *Saviryata Avadhi'* term is mentioned in context of the time period during which the *Virya* (potency) of any drug remains unaffected due to environmental/ microbial deterioration.

The shelf-life of *Taila* formulation was different according to different Acharya i.e. Sharangdhara (16 months), Yogaratnakara (12 months) and Vangasena (6 months)[2] while *Taila* preparation has shelf life of 3 year according to Gazette of India.[3] The trial drugs were prepared under standard operating procedure. Hence, in the present study on effort was taken to evaluate stability of oil with respect to microbial contamination in different climatic conditions. A baseline microbial profile was studied at regularly in particular intervals for total 15 months (from 26/10/2020 to 05/01/2022). Present aim of this article is to study the stability of *Panchavalkaladi Taila* and *Dashamoola Taila* at different climatic conditions

(temperature and humidity set ups) to rule out any microbial contamination.

MATERIALS AND METHODS

Drug Material

Jingini and *Dhava* were collected from the forest region of Taluka Una (Gir-Somnatha District), Gujarat. *Shallaki* was collected from the Gondal pharmacy, Gondal Dist. Rajkot, Gujarat. *Yavakuta* of *Dashamoola* was collected from the Pharmacy of Gujarat Ayurved University, Jamnagar. Identification and authentication of all procured raw drugs were done from Pharmacognosy laboratory of ITRA, Jamnagar before manufacturing. [Table 1 & 2]

Table 1: Contents of *Panchavalkaladi Taila*

S.No.	Drug	Botanical name	Part used	Ratio
1	*Vata*	*Ficus bengalensis* Linn.	Stem bark	1 part
2	*Udumbara*	*Ficus racemosa* Linn.	Stem bark	1 part
3	*Ashvattha*	*Ficus relliosa* Linn.	Stem bark	1 part
4	*Plaksha*	*Ficus lacor* Buch.	Stem bark	1 part
5	*Parisha*	*Thespesia populnea* Linn.	Stem bark	1 part
6	*Jambu*	*Syzygium cumini* Linn.	Stem bark	1 part
7	*Shallaki*	*Boswellia serrate* Roxb.	Stem bark	1 part
8	*Dhava*	*Anogeissus latifolia* Wall.	Stem bark	1 part
9	*Jingini*	*Odina woodier* Roxb.	Stem bark	1 part
10.	*Tila Taila*	*Sesamum indicum* Linn.	-	-

Table 2: Contents of *Dashamoola Taila*

S.No.	Drug	Botanical name	Part used	Ratio
1	*Bilwa*	*Aegle marmelos* Corr.	Stem Bark	1 part
2	*Agnimantha*	*Clerodendrum phlomidis* Linn.	Stem Bark	1 part
3	*Shyonaka*	*Oroxylum indicum* Vent.	Stem Bark	1 part
4	*Patala*	*Stereospermum suaveolens* DC.	Stem Bark	1 part
5	*Gambhari*	*Gmelina arborea* Linn.	Stem Bark	1 part
6	*Shalaparni*	*Desmodium gangeticum* DC.	Whole plant	1 part
7	*Prushniparni*	*Uraria picta* Desv.	Whole plant	1 part
8	*Bruhati*	*Solanum indicum* Linn.	Whole plant	1 part
9	*Kantakari*	*Solanum xanthocarpum* Schrad & Wendl	Whole plant	1 part
10	*Gokshura*	*Tribulis terrestris* Linn.	Fruit	1 part
11	*Tila Taila*	*Sesamum indicum* Linn.	-	-

Preparation of Drug

The drug was prepared in pharmacy of Gujarat Ayurved University, Jamnagar by adopting procedure given for oil preparation.

Date of preparation of *Panchavalkaladi Taila*: 01/08/2020

Date of preparation of *Dashamoola Taila*: 15/03/2020

Method of preparation of *Punarnavadi Guggulu*

Coarse powder was prepared from the raw material of *Panchavalkaladi* / *Dashamoola.* It was soaked in water overnight and *Kwatha* was prepared by boiling it to 1/8th.

A bolus was formed by adding sufficient amount of water in all *Kalka Dravyas* which were taken in *Choorna* form. *Tila Taila* was taken and heated to melt it. Then *Kalka Dravyas* were added in *Taila* followed by prepared *Kwatha*. It was boiled until *Siddhi Lakshana* were obtained.

Storage: Both oils were stored in stainless steel containers in a storeroom.

Methods

Microbial Profile

Microbial contamination was assessed by two methods (Smear examination and Culture study) 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:

Objective: To rule out any mycological findings

Specimen: 1. *Panchavalkaladi Taila* **2.** *Dashamoola Taila*

Procedure for Wet Preparation

A clean grease free glass slide was taken. Selected material was taken and distilled water was added. Grease free cover glass was covered and observed under the high power (40x) lens and reported as per findings.

Procedure for 10% KOH Preparation

Potassium Hydroxides pelletsin and distilled water was taken to prepare 10% of the same in clean glass tube & mix well. Take a clean grease free glass slide and put a drop of specimen and add freshly prepared 10% KOH then cover with grease free cover glass. Allow it to rest for 15-20 minutes to remove extra debris other than fungal particles. Observe under high power (40x) lens and report as per findings (if found).

B. Gram's stain test:

Gram staining differentiates bacteria into two groups i.e., Gram Positive Bacteria (GPB) and Gram Negative Bacteria(GNB). The gram stain procedure is based on the ability of microorganisms to retain color of the stains. GNB was decolorized by any organic solvent (acetone or Gram's decolorizer) while GPB were not decolorized as primary dye retained by the cell and was remain as purple. Further step of this decolorization, 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). [7]

Aim: To rule out any bacteriological findings in the specimen.

Specimen: 1. *Panchavalkaladi Taila* **2.** *Dashamoola Taila*

Procedure for Gram' Stain

A clean grease free glass slide was taken and prepared dry equal thick preparation (i.e. smear). Fixed prepared smear by passing 3-4 times over the flame of Bunsen burner (The fixation kills vegetative form of microbes and render them permeable to stain, make the material stick to the surface of slide & prevent autolytic changes). Then cover fixed prepared smear with Gram's crystal violet solution and allow to remain for mentioned time as per kit procedure. Washed off smear to remove excessive reagent with tap water than cover smear with Gram's Iodine solution and allow remaining for mentioned time as per kit procedure Washed off smear to remove excessive reagent with tap water. Decolorize smear with Gram's decolourizer by holding the slide at slope position and pour gram's decolourizer – acetone from its upper end up to removal of color of primary dye (i.e. Gram's Crystal Violet) or as per kit procedure. Washed off smear to remove excess acetone with tap water than cover smear with Safranin solution and allow remaining for mentioned time as per kit procedure. Smear was washed off with tap water to remove excessive reagent than was blotted and allowed to dry smear. Blot and allow to dry smear Examine under oil immersion lens and report as per findings (if found).

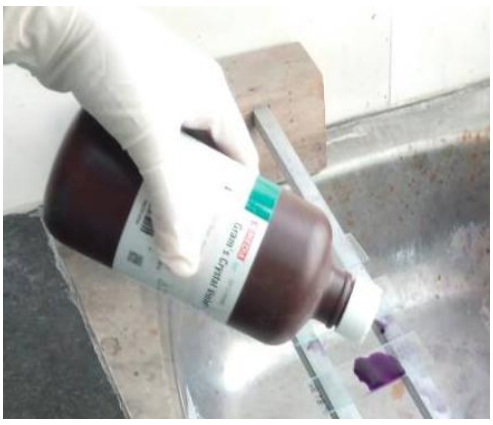

Figure 1 & 2: Smear staining Procedure

Culture Study

A. Fungal culture

Sample 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°C

Use of media: For selective cultivation of pathogenic fungi.

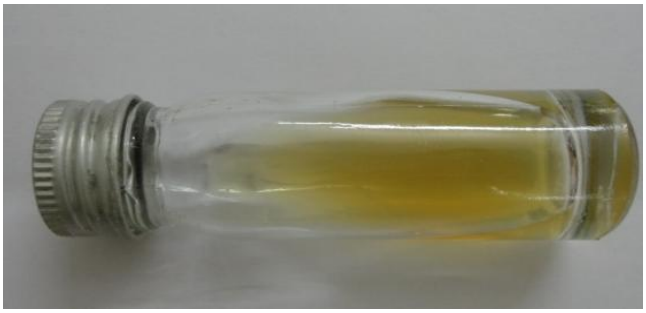

Figure 3: Sabouraud Dextrose Agar Base Media used for cultivation of any Fungal Contamination (SDA) bottle

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) by choosing appropriate selective solid media for inoculation purpose. Dry selective solid media in Hot Air Oven before specimen inoculation and allow it to cool dried medium before **Specimen inoculation Inoculate** selective specimen by Sterile cotton swab or by Nichrome wire (24 S.W.G. size) loop [In Bunsen burner, the first sterile loop oxidase flame-blue flame that was allowed cool after that loop was charged with selected specimen to be cultured. One loop full of the specimen is transferred onto the surface of well dried culture media] After inoculation/streaking process incubate inoculated medium in inverted position at 37^0 c for 05 to 07 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 (if found).

B. **Aerobic culture method:**

Sample of *Panchavalkaladi Taila* and *Dashamoola Taila* 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°C; Use of media: for selective cultivation of pathogenic bacteria.

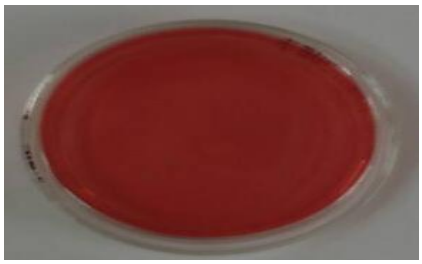

Figure 4.Mac Conkey Agar (MA)

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, then dry selective solid media in Hot Air Oven before specimen inoculation and allow it to cool dried before specimen inoculation. Selected specimen was inoculated by four flame method (the loop was flamed and cooled between the four sets of streaks) 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 loop full 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 that incubation period is examined the growth in form of colony and confirm growth by performing different related biochemical

reactions and different related staining procedures. After that reports the isolates (if found).

OBSERVATIONS AND RESULTS

Each and every time before giving to the patients, sample of *Panchavalkaladi Taila* and *Dashamoola Taila* were subjected to the microbiological study from the date of the preperation to the date of last consumption of *Panchavalkaladi Taila* and *Dashamoola Taila*. Observation and results are shown in table no. 3.

Table 3. Showing Observations of Sample of *Panchavalkaladi Taila* and *Dashamoola Taila* Preserved in Air Tight Container

Prepared batch	Date of investigations After preparation of the sample	Temp. (°C)	Humidity	Observations of Both Sample (Sample: 1. *Panchavalkaladi Taila* 2. *Dashamoola Taila*)			
				Gram's Stain	Aerobic Culture	Wet mount/ 10% KOH Preparation	Fungal Culture
1	03/11/2020	35°	26%	Microorganism not seen	No organisms isolated	Fungal filament not seen	No fungal pathogen isolated
2	02/12/2020	35°	29%	Microorganism not seen	No organisms isolated	Fungal filament not seen	No fungal pathogen isolated
3	04/01/2021	32°	32%	Microorganism not seen	No organisms isolated	Fungal filament not seen	No fungal pathogen isolated
4	03/02/2021	31°	30%	Microorganism not seen	No organisms isolated	Fungal filament not seen	No fungal pathogen isolated
5	04/03/2021	37°	22%	Microorganism not seen	No organisms isolated	Fungal filament not seen	No fungal pathogen isolated
6	06/04/2021	31°	42%	Microorganism not seen	No organisms isolated	Fungal filament not seen	No fungal pathogen isolated
7	11/05/2021	40°	26%	Microorganism not seen	No organisms isolated	Fungal filament not seen	No fungal pathogen isolated
8	02/06/2021	40°	30%	Microorganism not seen	No organisms isolated	Fungal filament not seen	No fungal pathogen isolated
9	05/07/2021	37°	44%	Microorganism not seen	No organisms isolated	Fungal filament not seen	No fungal pathogen isolated
10	18/08/2021	32°	55%	Microorganism not seen	No organisms isolated	Fungal filament not seen	No fungal pathogen isolated
11	07/09/2021	32°	73%	Microorganism not seen	No organisms isolated	Fungal filament not seen	No fungal pathogen isolated
12	06/10/2021	32°	59%	Microorganism not seen	No organisms isolated	Fungal filament not seen	No fungal pathogen isolated
13	10/11/2021	34°	14%	Microorganism not seen	No organisms	Fungal filament not	No fungal pathogen

					isolated	seen	isolated
14	06/12/2021	31°	29%	Microorganism not seen	No organisms isolated	Fungal filament not seen	No fungal pathogen isolated
15	05/01/2022	27°	58%	Microorganism not seen	No organisms isolated	Fungal filament not seen	No fungal pathogen isolated

DISCUSSION

Baseline microbial test must be conduct by every researcher to define conditions for stability testing for global level. For this, the many pharma companies are orienting their protocols to single set of conditions that covers extreme environmental conditions. A number of factors are used to assign the shelf life and these factors may also affect active pharmaceutical ingredients of drug. Moreover, testing under combination of three environmental factors i.e., temperature, humidity and light, has been reported to result in stronger deleterious effect on drug substances and products, than under temperature and humidity conditions only. [4,5] Total 15 test was done of both oils periodically with one month interval. Basic microbial assay that was conducted following the standard operating procedure revealed no microbial contamination in both oil during the clinical study. During this study, changes in temperature and humidity of environment was observed. Optimum temperature for microbial growth is temperature at which microbes multiplies i.e., psychrophilic bacteria (low temperate loving) are -20 to +10 °C while for mesophilic bacteria (moderate temperate loving) and thermophilic (high temperate loving) bacteria is 20-45 °C and 41-122 °C respectively.[6] The storage container and condition of each oil was maintained during study. The region where the drug was prepared and stored sample was very proximal to sea coast, this area has longest sea shore and maximum number of sea ports. Hence, relative humidity (RH) remains high in all the seasons throughout the year. Highest RH observed was 73 % in month of September while lowest relative humidity was 14% observed in month of November (as shown in Table 3). High RH may allow the growth of microbes [7],

Although air cannot be considered dry at RH more than 40%. 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 03/11/2020 to 05/01/2022. During this study period, any microbes were not isolated as a result of aerobic culture and any fungal pathogen were not isolated as a result of fungal culture (as shown in Table 3).

For long term storage, moisture content of drug plays a key role in sea coast area. Moisture contents also acts as an enzymatic activator which slowly decompose the drug resulting in its degradation as well as drug deterioration.[8] Moisture contents also affect the color of oil.[9] Any changes in color were not observed in both oils during study periods. Thus, there were not found any microbial growth during above period. Hence, *Panchavalkaladi Taila* and *Dashamoola Taila* could be safe in relative humidity, temperature and also within shelf-life period as mentioned in Gazettes of India.

CONCLUSION

Microbiological study of *Panchavalkaladi Taila* and *Dashamoola Taila* shows its stability during the period of 26th October 2020 to till last consumption i.e., 05th January 2022 for total of 16 months that shows its good shelf life.

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***Address for correspondence**
Dr. Matangee Pandya
Ph.D. Scholar,
Department of Prasutitantra Evum Streeroga,
Institute of Teaching and Research in Ayurveda, Jamnagar.
Email:
drmatangee144@gmail.com
Phone: 9106241594