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Original Article

Stability Study of *Panchendriyavivardhana Taila*, used in Treatment of Cerebral Palsy with respect to Microbiological Study

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ABSTRACT

Background: Cerebral Palsy is a neurological condition caused by brain damage and it is the most common motor and movement disability of childhood. In present study, *Acharya* Kashyapokta *Panchendriyavivardhana Taila* was used. 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. **Aim**: To carried out stability of *Panchendriyavivardhana Taila* with respect to its stability against microbial contamination. **Materials and Methods**: Sample of *Panchendriyavivardhana Taila* was prepared and studied to check microbial contamination at regular time intervals. **Results**: At the end of study *Panchendriyavivardhana Taila* container has not presence of microbes for 348 days of preparation sample, even in different climate and temperature. *Panchendriyavivardhana Taila* was stored in air tight steel container in during different climatric conditions and temperature. Sample was studied at regular intervals for a period of 348 days for to analyze mycological findings and presence of bacteriological findings by Wet mount preparation and Gram stain test respectively. Stability of sample was found at the 32˚C-44˚C range of temperature and 14%-27% of the humidity of environment at respective days. **Conclusion**: In the microbiological study of *Panchendriyavivardhana Taila*, there was no growth found of microorganisms from the 5th February 2019 till date 8th January 2020 i.e. 11 months & 3 days even in different climate and temperature. Hence in present study the stability test of *Panchendriyavivardhna Taila* with respect to microbiological findings was negative at room temperature, warm and cold, dry and humid conditions.

Keywords: Cerebral Palsy, Climate conditions, Microbial profile, *Panchendriyavivardhana Taila*, Stability.

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1. INTRODUCTION

Cerebral Palsy (CP) is a group of permanent movement disorders that appear in early childhood. Signs and symptoms vary among people and over time. Often, symptoms include poor coordination, stiff and weak muscles and tremors [1]. Exact correlation of the present condition cannot be made with any condition described in Ayurveda classics but some conditions found discrete in classics at different places like *Phakka, Pangulya, Mukatva, Jadatva, Ekanga Roga, Sarvanga Roga, Pakshaghata, Pakshavadha* etc. under the group of *Vata Vyadhi*.

In this context *Acharya* Kashyapa described *Panchendriyavivardhna Taila* in *Shatakalpadhyaya.* It is also known as *Panchbhoutika Taila* having the properties of improving power of all *Panchendriya* [2]. As in Cerebral Palsy motor functions were hampered that's why this *Panchendriyavivardhana Taila* was used for *Nasya*. The drug was prepared in pharmacy of Gujarat Ayurved University, Jamnagar by adopting standard operative procedure for *Taila* preparation. No any preservative was added to the test drug. Drug preparation was finished on 31/01/2019. Finished product was stored in airtight steel containers at room temprature.

The concept of *Virya* (potency) of the various dosage form of Ayurvedic is *Saviryata Avadhi*, which means that time period of active potency in drug [3]. It was necessary to prepare the formulation in a better form which is also free from microbial contamination. Stability of a pharmaceutical product is the capability of a particular formulation in a specific container or closure system, to remail within its physical, chemical, microbiological therapeutic specifications. Thus in the present study on attempt was taken to check stability of *Panchendriyavivardhana Taila* with respect to its Microbial profile at different climatic conditions and temperature setups at regular interval for a period of 08/01/2020.

Prepration of the *Taila*- The *Taila* was prepared by the method of *Sneha Kalpana Vidhi* as mentioned by *Acharya* Kashyapa in the description of this *Taila*. *Taila* was prepared in the pharmacy of GAU, Jamnagar. In this method *Tila Taila* will be taken followed by addition of *Kalka* on above mentioned drug. After than milk will add as per mentioned proportion (4 times). Then it was processed by heat up to *Samyaka Sidhhi Lakshana* of *Taila Paka*. Filter and stored in clean air tight container [2].

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

2. MATERIALS AND METHODS

Sample of *Panchendriyavivardhna Taila* was prepared (stored at room temperature) and finished product studied to check microbial contamination at regular intervals (upto drug used). Microbiological study has been carried out in Microbiology Laboratory, I.P.G.T. & R.A., 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 5th day of preparation of *Panchendriyavivardhana Taila*. Then samples from same container were subjected to the microbiological study regularly with random intervals during different seasons.

Drug material:

All the raw drugs were obtained from Pharmacy of Gujarat Ayurved University, Jamnagar. The ingredients, part used and their substitute drug are given in **[Table 1, 2]**.

Table 1: Ingredients of *Panchendriyavivardhana Taila*

Content	Botanical name	Part used	Ratio
Draksha	*Vitis vinifera* Linn.	*Shushka Phala*	1 part
Madhuka	*Glycrrhiza glabra* Linn.	*Shushka Moola*	1 part
Pippali	*Piper longum* Linn.	*Shushka Phala*	1 part
Bala	*Sida cordifolia* Linn.	*Shushka Moola*	1 part
Prapaundarika	*Nelumbo nucifera* Gaeris.	*Panchanga*	1 part
Brihati	*Solanum indicum* Linn.	*Shushka Moola*	1 part
Manjishtha	*Rubia cordifolia* Linn.	*Shushka Moola*	1 part
Twaka	*Cinnamomum zeylanicum* Breyn.	*Shushka Twaka*	1 part
Punarnava	*Boerhavia diffusa* Linn.	*Panchanga*	1 part
Anshumati	*Desmodium gengeticum* DC	*Panchanga*	1 part
Vidanga	*Embelica ribes* Burm.f.	*Shushka Phala*	1 part
Saindhava	*Rock salt*	-	1 part
Neel Kamal	*Nymphoea stellate*	*Panchanga*	1 part
Swadanshatra	*Tribulus terrastris* Linn.	*Shushka Phala*	1 part
Rasna	*Pluchea lanceolata* C.B.clarke	*Shushka Twaka*	1 part
Nidigdhika	*Solanum surratensa* Burm.f.	*Panchanga*	1 part
Tila Taila	*Sesamum indicum* Linn.	-	4 part
Godugdha	*Cow Milk*	-	16 part
Sharkara	*Sugar*	-	1 part

Table 2: Table 2 Substitute drug used in *Panchendriyavivardhana Taila*

Main Drug	Substitute	Botanical name of substitute drug	Part used	Ratio
Jivaka	*Vidarikanda*	*Puararia tuberosa* DC	*Shushka Kanda*	1 part
Rishabhaka	*Vidarikanda*	*Puararia tuberosa* DC	*Shushka Kanda*	1 part
Meda	*Shatavari*	*Asparagus recemosus* Willd	*Shushka Moola*	1 part

***Jivaka, Rishabhaka, Meda* are not available in present era, so their substitutes were used.**

Date of preparation:

Panchendriyavivardhana Taila was prepared on 31/01/2019.

Storage:

Finished product of *Panchendriyavivardhna Taila* was stored in air-tight, steel 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.

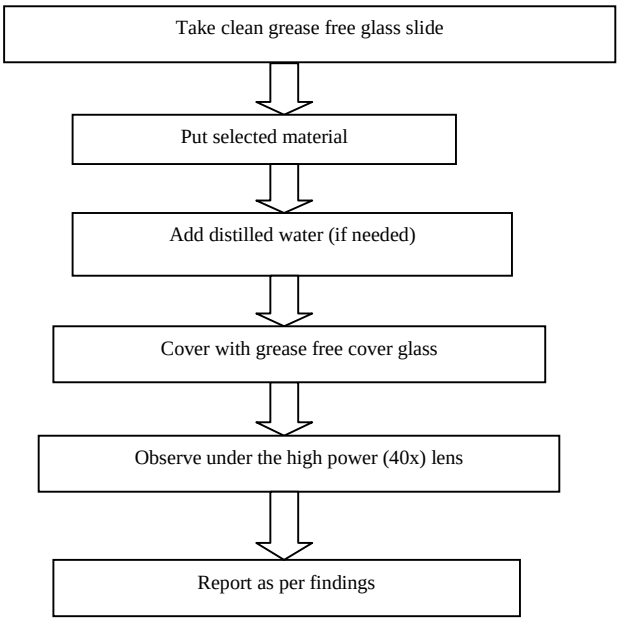
1. Smear Examination:

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

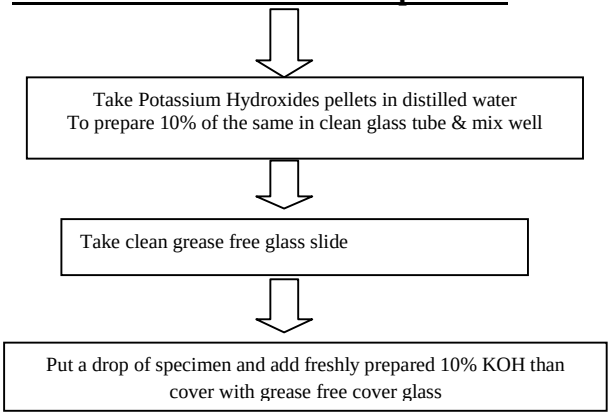
Aim: To rule out any mycological findings.

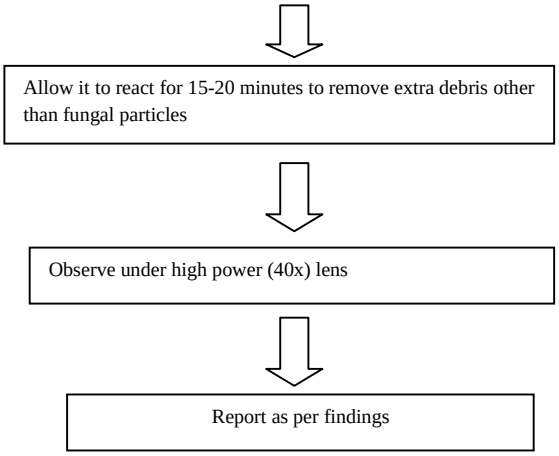
Specimen: *Panchendriyavivardhana Taila*

Procedure for Wet Preparation



Procedure For 10% KOH Preparation





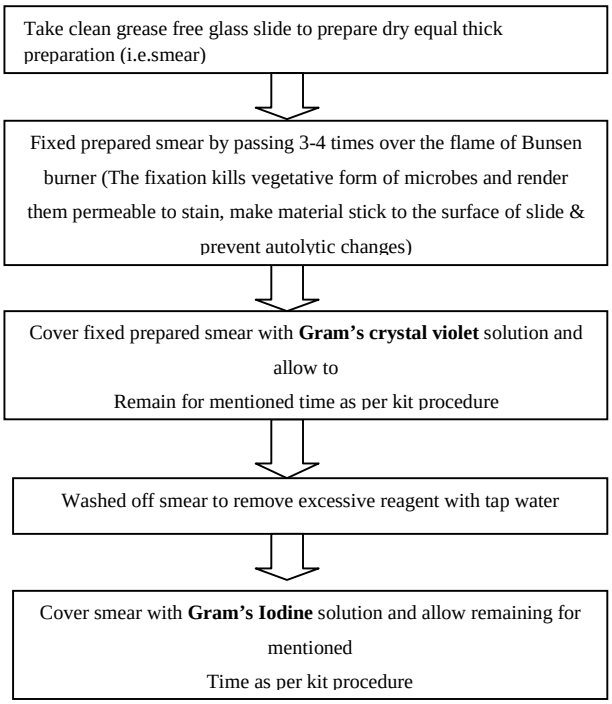
B. Gram's stain test:

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 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) [4].

Aim: To rule out any bacteriological findings.

Specimen: ***Panchendriyavivardhana Taila***

Procedure For Gram's Stain



Washed off smear to remove excessive reagent with tap water

Decolourize smear with **Gram's decolourizer** by holding the slide at slope position and pour gram's decolourizer – acetone from its

Washed off smear to remove excess acetone with tap water

Cover smear with **Safranin** solution and allow remaining for mentioned time as per kit procedure

Washed off smear to remove excessive reagent with tap water

Blot and allow to dry smear

Examine under oil immersion lens and report as per findings

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: Sabouraud Dextrose Agar Base (SDA), Modified (Dextrose Agar Base, Emmons)

Company: HIMEDIA Laboratories Pvt. Ltd.

Required time duration : 5 to 7 days

Required temperature : 37 ºC

Use of media: For selective cultivation of pathogenic fungi

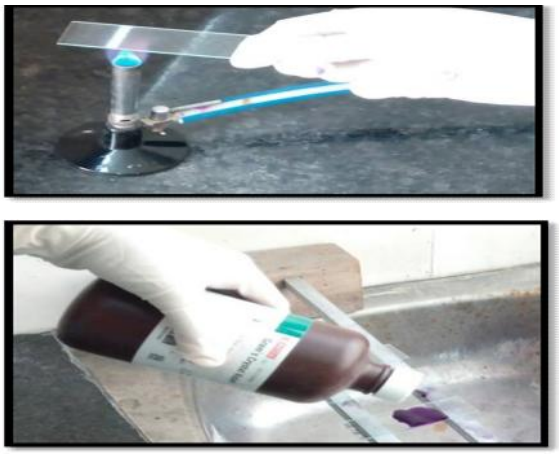


Fig 1, 2: Smear staining procedure

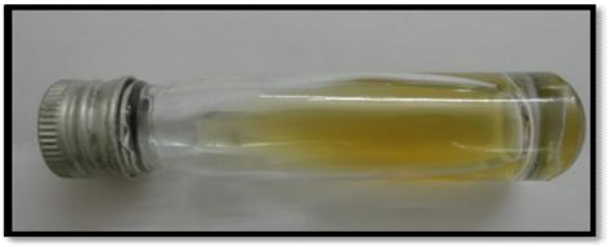


Fig 3: Sabouraud Dextrose Agar Base (SDA) bottle

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)

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 aliow 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 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 nacked eye in form of colony or arial growth and confirm growth by performing different related biochemical reactions and different related staining procedures .After that report isolates.

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.

Table 3: Showing observations of *Panchendriyavivardhana Taila*

Sr. No.	Days of study at	Date of investigations(After preparation of sample)	Temp. of Environment	Humidity of Environment	Observations of sample			
					Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
1	5 days	5th Feb 2019	32˚ C	16%	Microorganisms: Not seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
2	34 days	6th March 2019	32˚ C	17%	Microorganisms: Not seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
3	75 days	16th April 2019	32˚ C	24%	Microorganisms: Not seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
4	85 days	26th April 2019	44˚C	24%	Microorganisms: Not seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
5	128 days	3rd June 2019	44˚ C	27%	Microorganisms: Not seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
6	159 days	3rd July 2019	38˚C	27%	Microorganisms: Not seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
7	192 days	6th Aug 2019	32˚ C	25%	Microorganisms: Not seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
8	224 days	4th Sept 2019	32˚C	25%	Microorganisms: Not seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
9	254 days	4th Oct 2019	33˚ C	24%	Microorganisms: Not seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
10	386 days	6th Nov 2019	33 ˚C	23%	Microorganisms: Not seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
10	315 days	5th Dec 2019	33˚C	21%	Microorganisms: Not seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
11	348 days	8th Jan 2020	32˚C	14%	Microorganisms: Not seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated

Fig 4: MacConkey 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

↓

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 nacked eye in form of colony and **confirm growth** by performing different related biochemical reactions and different related staining procedures. After that **report** isolates.

Every time sample (in which drug preserved) were subjected to the microbiological study from the date of the preperation to the date of last microbiological study. Results are shown in table 3.

3. RESULTS AND DISCUSSION

Ayurveda as an adjuvant therapy is widely used in neurological disorders like Cerebral Palsy. The present Study was carried out to observe the stability study of *Panchendriyavivardhana Taila* with respect to Microbial Contamination of sample prepared and preserved in different climatic and temperature conditions. 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. According to *Acharya* Sharangdhara, *Saviryata* of *Taila* is 16 months [5]. Due to low environmental oxidation rate in the *Taila* preparation, *Saviryata* of *Taila* remains good in compare to the *Churna*. These are also remaining unaffected by microbial infestation until it develops some moisture which can facilitate the microbial growth.

Baseline microbial profile of *Pachendriyavivardhana Taila* was studied for 348 days. At the end of study *Panchendriyavivardhana Taila* was not found any microbes. Stability of *Panchendriyavivardhana Taila* was found at the range of temperature of environment at respective days was 32˚C-44˚C and the humidity of environment at respective days was 14%-27%.

4. CONCLUSION

Hence Microbiological study showed that the quality of *Panchendriyavivardhana Taila* is in a standard condition. There were no growth found of microorganisms (bacterial or fungal), till 08/01/2020 i.e. 348 days of *Panchendriyavivardhana taila* from the date of preparation, shows its good shelf life.

5. REFERENCES

1. Cerebral Palsy, https://en.wikipedia.org/wiki/Cerebral_palsy#cite_note-NINDS2013-1 (Accessed 20th july 2020).
2. Pandit Hemaraj Sharma ed, Kashyapa Samhita of Vrudhdhajivaka, (Sans)(Hindi), Kalpa Sthana, Shata Kalpa, 32/40, Chaukhambha Vishvabharati: Varanasi, 2015, pp. 279.
3. Shree Rasiklal Parikh ed, Sharangdhara Samhita, Purva Khanda, Paribhasha Adhyaya, 1/54-56, Sastu Sahitya Vardhaka Karyalaya, Mumbai, 2012, pp.13
4. Alfred E Brown, Benson: Microbiological Application, 8th Edition, the McGraw – Hill Companies, 2001, P. 64.
5. Shree Rasiklal Parikh ed, Sharangdhara Samhita, Purva Khanda, Paribhasha Adhyaya, 1/54-56, Sastu Sahitya Vardhaka Karyalaya, Mumbai, 2012, pp.13

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