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STABILITY STUDY OF DANTASHODHANA PASTE WITH RESPECT TO BASELINE MICROBIAL PROFILE

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ABSTRACT:

Introduction: Ayurveda the life of science gives guideline to maintain health with daily regimen. Oral health is an integral part of general health. *Dantasharkara* (Dental Calculus) is a disease which occurs due to poor oral hygiene. After removal of *Dantasharkara* importance must be given to the oral hygiene to prevent the recurrence. *Dantashodhana Choorna* mentioned in *Sushruta Samhita* can be used for this purpose. Toothpaste was prepared from this formulation. Microbial contamination should be taken care of to increase shelf life of any formulation. In the present study, stability with respect to microbial profile of *Dantashodhana* Paste was carried out. *Dantashodhana* Paste was prepared in the month of June 2016 and stored at room temperature. **Method**: *Dantashodhana* Paste was studied with respect to its microbial profile under different climatic conditions at regular interval of one month for duration of 8 months. To analyze mycological findings and presence of microorganisms, Wet mount test and Gram stain test where applied respectively. **Result**: At the end of the study, sample of *Dantashodhana* Paste did not reveal presence of microbes. **Conclusion**: The findings of the present study concluded that *Dantashodhana* Paste did not show any kind of microbial contamination with the changing in climatic conditions.

Keywords: Climatic, *Dantashodhana* Paste, Microbial Profile, Stability

INTRODUCTION: Importance of oral hygiene has been described by ancient sags of Ayurveda thousands years back and it was an imperative part of daily regimen. When routine oral hygiene is not maintained diseases of oral cavity (*Mukha Roga*) occurs. *Dantasharkara* is one of the *Mukha Roga*. *Dantasharkara* means collection of sand like particles i.e. tartar at the junction of teeth and gums and in between the teeth. The tartar is rough and hard and is often associated with halitosis.(i) *Dantasharkara* should be scraped without injuring the gums.(ii) *Dantashodhanaa Choorna* has been described by *Aacharya Sushruta* for maintaining the oral hygiene.(iii) This tends to cleanse the teeth and remove the bad smell from the mouth as well as the subdue *Kapha*. It cleanses the mouth and also produces a good relish for food and a cheerfulness of mind.(iv) Composition of *Dantashodhana Choorna* is as per mentioned in Table no. 1

Shelf life or *Saviryata Avadhi* is considered as 'best before use date' after which one or more properties of the formulation shows considerable

degradation. It has been mentioned for in our ancient texts very long back. But toothpaste is not a classical *Ayurvedic* drug form. It is need of time to determine shelf life for it. But now a days, paste form is more convenient and more acceptable for the patients than powder form. Contents of *Dantasodhana* paste are same as *Dantasodhana Choorna.* Common base of toothpaste was used to make *Dantasodhana* paste. *Dantasodhana* paste is administered as a paste for tooth brushing which should be free from any microbial and fungal contamination. Composition of *Dantashodhana* Paste is as per mentioned in Table no. 2

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.(v)

Microbial communities are extremely complex in structure and function and can be affected by climate and other global changes in many ways. Thus the present study was designed to study the stability of *Dantasodhana* paste with respect to microbial contamination.

MATERIALS AND METHODS:

Dantashodhana Paste was prepared and studied to check microbial contamination at different climatic conditions. The study was conducted at Microbiology laboratory, Institute for Post Graduate Teaching and Research in Ayurveda, Jamnagar, Gujarat, India.

Collection of the raw material: Raw herbal drugs and *Saindhava* were collected from Pharmacy, Gujarat Ayurved University, Jamnagar. Base for toothpaste was collected from local market.

Preparation of the *Dantashodhana* Paste: As specific method of preparation is not mentioned for this drug, Drugs used in *Dantashodhanaa Choorna* except *Saindhava*, honey and *Taila* were collected in *Yavakuta* form, *Kwatha* (Decoction) was prepared adding sixteen times water and reducing it to one fourth. After observing *Kwatha* (Decoction) *Sidhdhi Lakshana* it was filtered with dry and clean cotton cloth. *Kwatha* (Decoction) was then again subjected to heat to prepare *Ghana* (contiontrated aqueous herbal extract). After *Ghana* (contiontrated aqueous herbal extract) preparation *Ghana* (contiontrated aqueous herbal extract) and base materials for toothpaste were mixed in proportion of 30:70 respectively. Mixture was triturated properly in edge runner for one day until homogenous form was obtained. This paste was then filled in non-rigid collapsible tube containers of twenty grams each and sealed carefully.

Dantashodhanaa Paste was prepared and stored in July 2016.

Storage: *Dantashodhana* Paste was stored at room temperature in safe place.

Selection: Sample is selected randomly for study of microbial contamination for 8 months.

Microbial profile: Microbial contamination was assessed by two methods to check any mycological findings and bacteriological findings. The details of the procedures followed are given below.

Aims: To rule out any fungal and bacteriological findings.

Specimen: *Dantashodhana* Paste

Material: Samples of *Dantashodhana* Paste were prepared and studied to check

microbial contamination at regular interval of one month. The study was conducted at Microbiology laboratory, Institute for post graduate teaching and research in Ayurveda, Jamnagar, Gujarat.

Storage: *Dantashodhana* Paste was stored in room temperature.

Methods: Wet mount, Fungal Culture, Gram stain and Aerobic Culture tests were used to study microbial and fungus contamination in the samples. The sample *Dantashodhana* Paste was randomly selected to analyze mycological findings and presence of microorganisms. The study was conducted at Microbiology laboratory, IPGT &R A, Jamnagar. Different climatic and temperature conditions were studied at regular interval of one month for period of 8 months.

Microbial profile: Microbial contamination was assessed by two methods to check any mycological findings and bacteriological findings. The details of the procedures followed are given below.

1. Wet mounts test and fungal culture:

Aim: To rule out any mycological findings.

Specimen:*Dantashodhana* Paste

Procedure:

Smear examination: A drop of selected samples were taken on grease free glass slides + 10% KOH and covered with clean cover slips for microscopic examination.

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.

2. Gram 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 decolorized) 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. (vi)

Aim: To rule out any bacteriological findings.

Specimen: *Dantashodhana* Paste

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 decolorized 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 saffranin 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.

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 Coulmbia 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.

Results: Randomly selected sample of *Dantashodhana* paste was stored at room temperature did not show presence of any mycological or bacteriological contamination on wet mount test & fungal culture and gram stain test at the end of 8 months after preparation of the sample.

DISCUSSION: Drug should always be free of microbial contamination for better efficacy and longer storage. Necessary care was taken in pharmaceutical preparation and packaging to fulfill this purpose. Stability is usually expressed in terms of shelf life. The factors which may be considered when determining whether a prepared product requires time/temperature control during storage, distribution, sale and handling may be categorized under intrinsic, extrinsic and other factors (FDA report, 2001). Intrinsic factors include moisture content, pH and acidity, nutrient content, biological structure, redox potential, naturally occurring and added antimicrobials. Extrinsic factors include types of packaging/atmospheres, effect of time/temperature conditions on microbial growth, storage/holding conditions and processing steps (FDA report, 2001). Microbial growth should be avoided to increase its stability period and drug can be stored normally.

Dantashodhana Paste was prepared and stored in room temperature. Sample was selected randomly for study of microbial contamination. Study period covered almost all periods of year with highest climatic variation. Changes in temperature and humidity were may be at maximum during whole duration. In this study highest and lowest temperature observed were 38°C in the month of February and 11°C in January respectively. (Table 3) Average 26.125°C during whole study period. Optimum temperature for bacterial growth is temperature at which bacteria multiplies. This optimum temperature for bacteria For psychrophilic bacteria (cold loving bacteria) it is 15-20°C while for mesophilic (middle living) and thermophilic (heat loving) bacteria it is 30-37°C and 50-60°C respectively.(vii) Optimum temperature for psychrophilic and mesophilic bacteria falls in range of temperature observed during period of study. The region where the drug was prepared and sample was stored is very proximal to sea coast, this part of state has longest sea shore and most number of sea ports. So relative humidity (RH) remains high in almost all seasons of year. Highest RH observed was 100% while lowest was 12%. (Table 4) High RH can allow the growth of microbes.(viii) Relative humidity remained constantly high during study duration except month of

November and January when RH was low in comparison to other months, although air can not be considered dry at RH more than 40%.

Wet mount, Fungal culture, Gram stain and Aerobic culture tests were used to study microbial and fungus contamination in the samples every month from July 2016 to February 2017. During this study period no any organisms were isolated for aerobic culture**.** No Fungal Pathogen Isolated for fungal culture and no any microorganism were seen. [Table 5] Sample which was stored in condition open to all climatic changes did not showed any kind of microbial contamination. These results may contribute to some properties of formulation. Moisture content of formulation plays most important role. Naturally it is desired that the moisture content should be minimum, which will help, in long storage of the product. Moisture content is the main causative factor in product deterioration. Moisture in a product is sufficient to activate different enzymes, which slowly decompose the product resulting in its degradation.(ix) Although Ghana used in preparation of formulation is aqueous extract moisture content found on testing was low, which may be the cause for results. Herbal components used in formulation are having anti-microbial activity, for e.g. *Tejovati*.(x) This property of ingredients may played role in inhibition of microbial contamination.

CONCLUSION: Stability of a pharmaceutical product depends largely on many factors. Randomly selected samples of *Dantashodhana* Paste showed negative finding for bacterial as well as mycological contamination. The product is free from microbial contamination revealing its safety aspects. As no published Microbial profiles of *Dantashodhana* Paste are available; current observations can be considered as standard for future studies. More studies should be carried out on tooth pastes made with different formulation to determine a common standard for herbal tooth pastes.

Table 1: Composition of *Dantashodhana Choorna.*

Sr. No.	Name	Botanical / English name	Part used	Praportion
1.	*Vyosha* ✓ *Shunthi* ✓ *Maricha* ✓ *Pippali*	 *Zingiber officinale* Rose. *Piper nigrum* L. *Piper longum* L	 Rhizome Fruit Fruit	1 part
2.	*Trivarga* ✓ *Tvak* ✓ *Ela* ✓ *Tamala*	 *Cinnamomum zeylanica* Bl. *Elettaria cardamomum* Maton. *Cinnamomum tamala* Nees.	 Bark Fruit Bark	1 part
3.	*Tejovati*	*Zanthoxylum armetum* DC.	Bark	1 part
4.	*Saindhava*	Rock salt	-	1 part

Table 2: Ingredients of *Dantashodhana* Paste:

No.	Name	Percentage
1	*Dantashodhana Ghana*	27 %

2	*Dantashodhana Kwatha*	18 %
3	*Saindhav*	7.20 %
4	Glycerin	12.60 %
5	Sorbitol	12.60 %
6	CMC	1.26 %
7	SLS	3.24 %
8	Calcium Carbonate	18 %
9	Methylparaben	0.02 %
10	Propylparaben	0.02 %
11	Saccharine	0.06 %
12	Peppermint oil	0.5 %

Table-3: High and low weather summery of temperature during study period for *Dantashodhana* Paste sample: (xi)

Months	**Temperature**				
	High	**Date & time**	**Low**	**Date & time**	**Average**
July- 2016	34°C	6th July 17:30	25°C	31st July 20:30	29°C
August- 2016	33°C	18th August 14:30	24°C	24th August 2:30	28°C
September-2016	35°C	15th September 14:30	24°C	27th September 5:30	28°C
October-2016	35°C	16th October 14:30	21°C	31st October 5:30	28°C
November-2016	35°C	1st November 14:30	17°C	20th November 5:30	26°C
December-2016	33°C	7th December 14:30	14°C	27th December 5:30	24°C
January-2017	33°C	24th January 14:30	11°C	11th January 8:30	21°C
February-2017	38°C	18th February 14:30	13°C	6th February 5:30	25°C

Table: 4 High and low weather summery of Relative Humidity during study period for *Dantashodhana* Paste sample: (xii)

Months	**Relative Humidity**				
	High	**Date & time**	**Low**	**Date & time**	**Average**
July- 2016	100%	12th July 17:30	47%	31st July 17:30	80%

August- 2016	100%	5th August 8:30	47%	24th August 14:30	84%
September-2016	98%	15th September 2:30	44%	27th September 17:30	75%
October-2016	100%	4th October 23:30	19%	31st October 11:30	67%
November-2016	98%	6st November 14:30	15%	2th November 14:30	45%
December-2016	100%	24th December 5:30	13%	25th December 14:30	48%
January-2017	100%	1th January 5:30	13%	13th January 14:30	49%
February-2017	100%	1th February 8:30	12%	6th February 11:30	41%

Table-5: Observation of Sample *Dantashodhana* Paste preserved at room temperature

Sr. No.	**Months of investigations After preparation of the sample**	**Observation of sample**				**Temperature**	
		Gram's Stain	**Aerobic culture**	**Wet mount/ 10% KOH Preparation**	**Fungal culture**	**High**	**Low**
1.	July (25/7/2016)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated	30°C	27°C
2.	August (26/8/2016)	Microorganisms Not Seen	No organisms Isolated	Fungal filaments not seen	No Fungal Pathogen Isolated	26°C	27°C
3.	September (15/9/2016)	Microorganisms Not Seen	No organisms Isolated	Fungal filaments not seen	No Fungal Pathogen Isolated	35°C	26°C
4.	October (18/10/2016)	Microorganisms Not Seen	No organisms Isolated	Fungal filaments not seen	No Fungal Pathogen Isolated	34°C	24°C
5	November (15/11/2016)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated	31°C	19°C
6	December (13/12/2016)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated	30°C	19°C
7	January (12/01/2017)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated	25°C	13°C

8	February (15/2/2017)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated	36°C	23°C

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