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STABILITY OF *MADHU-GHRITA YOGA* AND *SWARNA PRASHANA YOGA*- A MICROBIAL STUDY

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ABSTRACT

Microbial testing has a wide variety of uses and thus, is an incredibly complex field of study. Microbial contaminations of products may ultimately contribute to secondary bacterial infections and lead to serious clinical hazards. This study therefore is aimed to determine the microbial contamination of *Madhu- Ghrita -Yoga* and *Swarna Prashana Yoga* with respect to microbial findings in the three selected samples of each groups prepared and stored in different climatic conditions and temperature. Microbial contamination was assessed by Wet Mount test / 10% KOH Preparation, Gram Stain test, Aerobic culture and fungal culture to check mycological and bacteriological findings. Negative findings of any microbial and fungus formation in all samples showed that prepared product with maintaining the hygiene condition, kept in ideal packages, preserved under normal temperature and dry air (below normal degree of humidity), increase the shelf life of these formulations.

KEYWORDS: Aerobic culture, fungal culture, microbial findings, *Madhu-Ghrita Yoga*, *Swarna Prashana Yoga*.

INTRODUCTION

Microbiology laboratories play a critical role in clinical, research, and industrial processes and organizations. Microbial testing has a wide variety of uses and thus, is an incredibly complex field of study. Samples may be cultured and tested for a variety of reasons from ensuring public safety by measuring the number of microbes in food or the environment, to aiding in the diagnosis of a disease and determining a course of treatment.[1]

Swarna Prashana is a comprehensive *Rasayana Chikitsa*, administered for the physical, mental, intellectual and spiritual wellbeing of the children. It is mentioned in Kashyapa Samhita. *Medha* (memory), *Agni* (improves the digestive power), *Bala* (strength), *Aayushya* (life span), *Mangalakara* (auspicious), *Punya* (virtuous), *Vrushya* (aphrodisiac), *Varnya* (fair complexion), *Grahapaha* (voids away associated evils) are developed in child after *Swarna Prashana*.[2] *Swarna Prashana Yoga* is prepared with *Swarna Bhasam*, *madhu-ghrita* with and without herbs. It is made as a form of suspension. Oral liquid drug formulations such as aqueous solutions, suspensions, emulsions and syrups used for pediatrics are at a greater risk of microbial contamination during consumption due to sweetening agents, reconstitution methods, improper storage and handling defects. Microbial contaminations may ultimately contribute to secondary bacterial infections and lead to serious clinical hazards particularly in children.

It is essential to determine the microbial contamination of all products whether sterile or non-sterile to ensure good quality of the product. This study therefore is aimed to determine the microbial contamination *Swarna Prashana Yoga* with respect to microbial findings in the three selected samples of each groups prepared and stored in different climatic conditions and temperature.

MATERIALS AND METHODS

Materials

Table 1: Details of Raw materials

Sr. No.	Material Name	Company Name	Packed Month	Expiry Month
1.	*Ghrita* (G)	Schreiber Dynamix Dairies Ltd., Maharastra	May 2015	Jan 2016
2.	*Madhu* (M)	Azad Kutir Udhyoga Sansthan, Uttarpradesh	May 2015	September 2016

Table 2: Details of Finished prepared materials

Sr. No.	Batch No.	Material	Date of Preparation	Climate	
				Tem.	Humidity
1.	**1**	*Madhu- Ghrita Yoga* (A)	20/06/2015	33^0C	57%
		Madhu- Ghrita-Swarna Yoga (B)	30/06/2015	32^0C	58%
		Madhu- Ghrita-Swarna-Vacha Yoga (C)	03/07/2015	32^0C	60%
2.	**2**	*Madhu- Ghrita Yoga* (A1)	04/02/2016	27^0C	20%
		Madhu- Ghrita-Swarna Yoga (B1)	06/02/2016	24^0C	54%
		Madhu- Ghrita-Swarna-Vacha Yoga (C1)	08/02/2016	26^0C	19%
3.	**3**	*Madhu- Ghrita Yoga* (A2)	21/09/2016	30^0C	71%

		Madhu- Ghrita-Swarna Yoga (B2)	14/09/2016	32^0C	59%
		Madhu- Ghrita-Swarna-Vacha Yoga (C2)	15/09/2016	34^0C	50%

❖ **Methods**

Preparation method

242 g of *Madhu* (honey) and 22g of *Ghrita* for sample A, 242 g of honey and 22g of *Ghrita* with 1gm of *Swarna Bhasma* (Ash of gold) and 242 g of honey, 22g of *Ghrita*, 1gm of *Swarna Bhasma* (Ash of gold) with 2 gm of *Vacha Churna* were, triturated well for about 6-8 hours in *Akika Khalvayantra* (Mortar and pestle made of semi- precious stone, *Akika*). This specific proportion was followed to fix the dosage form as drops which will be easier in administration in children. *Swarna Bhasma* was procured from the Department of *Rasashastra* and *Bhaishajya Kalpana*, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India. Honey (Golden Honey) of Azad Kutir Udhyoga Sansthan and Ghee (Dynamix Pure Cow Ghee) of Schreiber Dynamix Dairies Ltd. were procured from standard local market which was also evaluated for microbial contamination before the preparation of *Madhu-Ghrita, Swarna yukta Madhu-Ghrita* and *Swarna-Vacha yukta Madhu-Ghrita (Swarna Prashana Yoga).*

Figure 1*: Madhu-Ghrita* **Figure2: *Madhu-Ghrita-Swarna Yoga*** **Figure3: *Madhu-Ghrita-Swarna-Vacha Yoga***

Preparation time

Swarna Prashana Yogas were prepared in three different batches. The first batch samples (A, B, C) were prepared and preserved during the months of May to October, 2015, the second batch samples (A1, B1, C1) were prepared and preserved during the months of February to June, 2016 and the third batch samples (A2, B2, C2) were prepared and preserved during the months of August to October, 2016. The preparation was carried out with utmost care to avoid any sort of contamination by using sterile vessels and gloved hands. To ensure further

safety, the glass bottles used for storage of the samples were sterilized by boiled water and then kept in hot with sunlight.

Storage

Samples A, B, C, A1, B1 and C1 were stored in refrigerator. Samples A2, B2 and C2 were stored at room temperature in a dry and dark place to avoid exposure to direct sunlight and wind. No preservatives were added to the samples for storage.

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

1. **Smear examination**

(a) Gram stain test

(b) 10% KOH Preparation/wet mount test

2. **Culture**

(a) Aerobic culture

(b) Fungal culture.

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 colour of the stains used during the gram stain procedure. Gram-negative bacteria are decolorized by any organic solvent, losing the colour of the primary stain. Gram-positive bacteria are not decolorized and will remain as purple. After decolourization step, a counter stain is used to impart a pink colour to the decolorized gram-negative organisms. The Gram stain procedure enables bacteria to retain colour of the stains, based on the differences in the chemical and physical properties of the cell wall.[3]

Aim: To rule out presence of bacterial finding.

Specimen: Sample G, M, A, B, C, A1, B1, C1, A2, B2 and C2.

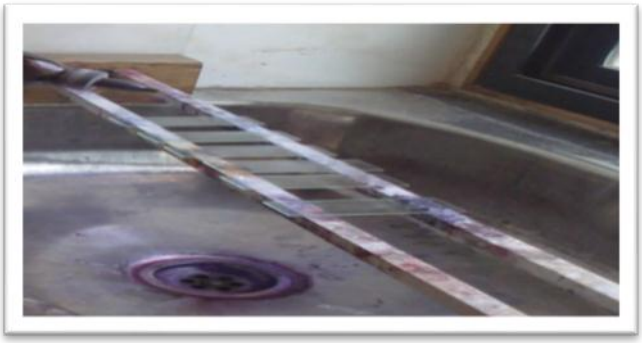

Figure 4: Gram stain

Procedure

- Take clean grease free glass slide to prepare 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 material stick to the surface of slide & prevent autolysis changes)
- Cover fixed prepared smear with Gram's crystal violet solution and allow to remain mentioned time as per kit procedure
- Washed off smear to remove excessive reagent with tap water
- Cover smear with Gram's Iodine solution and allow to remain for mentioned time as per kit procedure
- Washed off smear to remove excessive reagent with tap water
- Decolorize smear with Gram's decolorizer by holding the slide at slope position and pour gram's decolorizer –acetone from its upper and 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
- Cover smear with Safranin solution and allow to remain for mentioned time as per kit procedure
- Washed off smear to remove excessive reagent with tap water
- Biot and allow to dry smear
- Examine under oil immersion lens and report as per findings.

Wet mount test[4]

A glass slide holding a specimen suspended in a drop of liquid for microscopic examination.

Aim: To rule out any mycological findings.

Specimen: Samples G, M, A, B, C, A1, B1, C1, A2, B2 and C2.

Procedure

- Take clean grease free slide
- Put selected material
- Add distilled water
- Cover with grease free cover glass
- Observe under high power (40x) lens
- Reports as per findings

10% KOH Preparation

Potassium hydroxide (KOH) preparation is used for the rapid detection of fungal elements in clinical specimen, as it clears the specimen making fungal elements more visible during direct microscopic examination.[5]

Aim: To rule out any mycological findings.

Specimen: Samples G, M, A, B, C, A1, B1, C1, A2, B2 and C2.

Procedure

- Take potassium Hydroxides pellets (Himedia Lab. Pvt. Ltd.) in distilled water to prepare 10% of the same in clean glass tube & mixed well
- Take clean grease free glass slide
- Put a drop of specimen and add freshly prepared 10% KOH than cover with grease free cover glass
- Allow it to react for 15-20 minutes to remove extra debris other than fungal particles
- Observe under high power (40x) lens
- Report as per findings.

Aerobic Culture[6]

This test is used to isolate and identify potentially pathogenic aerobic organisms.

Aim: To rule out any mycological findings.

Specimen: Samples G, M, A, B, C, A1, B1, C1, A2, B2 and C2.

Procedure

- In the clinical microbiology laboratory culture method are employed for isolation of organisms.

(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 streaks i.e. four times on surface of cool dried medium with nichrome wire (24 S.W.G. size) loop first sterile loop in Bunsen burner oxidase flame blue and allow it to cool than loop is charged with selected specimen to be cultured. One loop of the specimen is transferred into the surface of well dried plate
- After inoculation/streaking process incubate inoculated medium in inverted position at 370c for 18 to 24 hrs in incubator under aerobic or 10% CO_2
- 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.

Fungal culture

Aim: To rule out presence of fungal finding.

Specimen: Sample G, M, A, B, C, A1, B1, C1, A2, B2 and C2.

Procedure

- 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 inoculationInoculate selected specimen by sterile cotton swab or by nichrome wire (24 S.W.G. size) loop first sterile loop in Bunsen burner oxidase flame blue and allow it to cool than loop is charged with selected specimen to be cultured. One loop of the specimen is transferred into the surface of well dried culture media

- After inoculation/streaking process incubate inoculated media in inverted position at 370c for 05-07 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.

RESULTS AND OBSERVATIONS

The following findings were observed at the end of the study. Observations of the samples during the tests at different intervals (i.e. 1 month) are presented below in tables 2, 3 and 4 respectively.

Table 3: 1st batch Samples (A, B and C) preserved in refrigerator

Sr. No.	Months of sample test	Observation			
		Gram Stain test	Aerobic culture	Wet Mount test / 10% KOH Preparation	Fungal culture
1.	May 2015	Microorganisms not seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen isolated
2.	June 2015	Microorganisms not seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen isolated
3.	July 2015	Microorganisms not seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen isolated
4.	Aug. 2015	Microorganisms not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen isolated
5.	Sep. 2015	Microorganisms not seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen isolated
6.	Oct. 2015	Microorganisms not seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen isolated

Madhu- Ghrita Yoga (Sample A) and *Swarna Prashana Yoga* (Sample B & C) preserved in refrigerator during the month of May to October 2015 did not show positive positive findings (mycological and bacteriological) at the end of 6 months after preparation of the sample.

Table 4: 2nd Batch Samples (A1, B1 and C1) preserved in refrigerator

Sr. No.	Months of sample test	Observation			
		Gram Stain test	Aerobic culture	Wet Mount test / 10% KOH Preparation	Fungal culture
1.	Feb. 2016	Microorganisms not seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen isolated
2.	March 2016	Microorganisms not seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen isolated

3.	April 2016	Microorganisms not seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen isolated
4.	May 2016	Microorganisms not seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen isolated
5.	June 2016	Microorganisms not seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen isolated

Madhu- Ghrita Yoga (Sample A1) and *Swarna Prashana Yoga* (Sample B1 & C1) preserved in refrigerator during the month of February to June 2016 did not show positive findings (mycological and bacteriological) at the end of 6 months after preparation of the sample. But *Swarna Prashana Yoga* (Sample C1) showed bacteriological and fungal findings so, it was discarded. So, another batch *Swarna Prashana Yoga* (Sample C1) was prepared and studied.

Table 5: 3rd Batch Samples (A2, B2 and C2) preserved in room temperature.

Sr. No.	Months after preparation of the sample	Observation			
		Gram Stain test	Aerobic culture	Wet Mount test / 10% KOH Preparation	Fungal culture
1.	Aug. 2016	Microorganisms not seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen isolated
2.	Sep. 2016	Microorganisms not seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen isolated
3.	Oct. 2016	Microorganisms not seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen isolated

Madhu- Ghrita Yoga (Sample A2) and *Swarna Prashana Yoga* (Sample B2 & C2) preserved at room temperature during the month of August to October 2016 did not show positive findings(mycological and bacteriological) at the end of 6 months after preparation of the sample.

DISCUSSION

Contamination of products can occur at any stage of production, processing, marketing and administration. Administration of contaminated pharmaceutical products can be harmful to the recipients such as, young and elderly patients. Survival and growth of microorganisms can deteriorate the product quality and production of metabolites/toxins may be harmful to the patient even they are present in minute quantities.

The classical descriptions of *Swarna Prashana* therapy uses metallic gold- rubbed on a clean rubbing stone with water, till fine gold particles are released. The rubbed gold or gold powder mixed with fine powder of *Medhya, Rasayana* herbs, *ghee* and honey is given to the

newborn. Rubbed gold in metallic form carries the risk of toxicity. Hence, a safer option of *Swarna Bhasma* is being used for the *Swarna Prashana* now a day. So, using *Swarna Bhasma* along with *Ghrita* and *Madhu* to achieve a fine colloidal suspension.

The growth of microorganisms is influenced by various physical factors i.e. temperature, moisture, pH, osmotic pressure, hydrostatic pressure, radiation and chemical factors i.e. oxygen, nitrogen, carbon, phosphorus, sulfur etc. of their environment.[7] All microorganisms need food. Sucrose sugar is best nutrient for microorganism growth. Honey's composition is about 38.5% fructose and 31% of glucose, 7.1% of maltose and 1.3% of sucrose.[8] Percentage of sucrose is less in honey, therefore fungus cannot grow. Honey works as a preservative because the high concentration of sugar in honey forces the water out of any fungus or bacteria cells that could otherwise contaminate the food. Eventually, the process of osmosis destroys those cells by drawing out all their water in other words, by drying them up. So because of its high sugar concentration, pure honey will never ferment or go bad.[9] The proportion of honey in these formulations is more than other contents, so the formulations are free from microbial contamination.

Very high and very low temperatures both obstruct the enzyme processes microorganisms depend on to survive, but individual species of microorganisms have grown to prefer different levels of temperature. The free flow of water is vital to microorganisms for their cells to exchange materials and for their metabolic processes. All microorganisms require some level of water, but a few can survive in low-moisture conditions by conserving all the water they find and by staying in a moisture-rich environment. As a general rule, though, the more moisture, the more microorganisms there will be found.[10] Negative findings of any microbial and fungus formation in all samples of three batches at room temperature as well as at refrigerator, during the month May 2015 to Oct. 2015, Feb. 2016 to June 2016 and August 2016 to October 2016 which may be due to low degree of temperature, humidity and the flow of moist wind and also depends upon proper hygienic conditions during preparation and storage. Temperature should not exceeding 30^0 C for oral drops and suspensions[11] and humidity permitting growth of molds varies from 75 to 95 per cent for different species.[12] Thus during the year of 2015, average 30^0C temperature and 67.5 % humidity and in 2016 average 30^0C temperature and 75 % humidity of Jamnagar were recorded.[13]

In one study, *Madhu- Ghrita Yoga* (1^{st} batch) showed positive finding in wet mount test only on 72^{nd} day of preparation when preserved in room temperature during the month of April-

June and *Madhu- Ghrita Yoga* on 30th day and *Madhu-Ghrita-Swarna Prashana Yoga* on 37th day (2nd batch) stored at room temperature during the months of September to November showed positive findings in gram stain test on. In both wet mount and Gram stain tests on 86th day of preparation showed positive findings in *Madhu- Ghrita Yoga* and *Madhu-Ghrita-Swarna Prashana Yoga* (2nd batch) stored at refrigerator. The month of September in 2013 received heavy rain fall in Jamnagar district and during October to December 2013 received average 27% higher rainfall in Jamnagar district. The low temperature, high humidity and moisture content of the atmosphere during these months might have influenced the earlier positive findings of microbial contamination in the samples of those periods.[14]

So that the level of microorganisms in the air is controlled by degree of humidity, temperature, air velocity and resistance of microorganisms to drying. Generally dry air and higher temperature has low microbial level. It is scientific truth that the decomposition or formation of fungi (fungus) is directly proportional to degree of humidity present in the atmosphere. The degree of humidity depends on natural rainfalls and the flow of moist winds.

CONCLUSION

This study reveals that *Madhu-Ghrita Yoga* and *Swarna Prashana Yoga* are prepared with maintaining the hygiene condition, kept in ideal packages, preserved under normal temperature and dry air (below normal humidity), so the shelf life of these formulations is increased.

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