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STABILITY STUDY OF MODIFIED MUSTA-TRIPHALADIAVALEHA WITH RESPECT TO BASELINE MICROBIAL PROFILE USED IN THALASSEMIA MAJOR

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ABSTRACT

Introduction: stability study of Modified *Musta- Triphaladi Avaleha* was done for 12 months (1 year) to know the stability of formulation in reference of its phyto-constituents and microbial growth therein. Modified *Musta-Triphaladi Avaleha* have been used in Thalassemia children as an adjuvant therapy prepared with various herbal product. **Methods:** Modified *Musta-Triphaladi Avaleha* was made in Pharmacy with standard classical method (*Avaleha Kalpana*) as per AFI part-1 (The Ayurvedic formulary of India, part-1 part- A) In present study, stability with respect to its Microbial profile of Modified *Musta-Triphaladi Avaleha* carried out. *Avaleha* was stored in 2 plastic containers during different climatic condition. *Avaleha* were studied at regular intervals for a period of 1 month to analysis Mycological findings and presence of Microorganisms by wet mount preparation and Gram stain test respectively. **Results:** At the end of study both *Avaleha* container has not present of microbes after 1 year of preparation, even in different climate and temperature. **Conclusion:** Since it is traditional *Avaleha kal-pana*, the shelf life period was about one year (Sharangdhar Samhita) The presented study reflects that Microbio-logical findings of Modified *Musta-Triphaladi Avaleha* was negative at room temperature, warm and cold, dry and humid condition.

Keywords: Microbial profile, Modified *Musta-Triphaladi Avaleha*, Climate condition, Stability.

INTRODUCTION

Thalassemia is a monogenic disorder characterized by abnormal synthesis of hemoglobin due to defects in the globin chain. This causes early excessive destruction of red blood cells leading to hypochromic, microcytic anemia, In Ayurvedic authentic texts, it cannot be found a disease similar to Thalassemia. But the methodology of understanding the unknown disease has been mentioned in *Charaka Samhita*[1] based on *Aaptopadesha Pramana* (Authoritative instruction). An effort has been made to correlate Thalassemia major with *Beejadushtijanya Pandu.*(Thalassemia Major) An ideal drug to treat Thalassemia should have *Deepana* (Appetizer), *Aamapachana* (Digestion of immature juice of food), *Srotovishodhana* (Opening of circulating micro channels), *Tridoshahara, Rochana* (Relish), *Jwarahara* (Antipiretic), *Pittasaraka* (*Pitta* excretor), *Lohashodhana* (Iron purifier from body), *Lohamarana* (Iron Cheltor), *Lekhana* (Scraping), *Bhedana* (Purgative), *Raktashodhana* (Blood Purifier), *Raktaprasadana* (Blood nutritive), *Shonitasthapana* (Haemostasis), *Varnya* (Glowing body complexion), *Balya* (Strengthen Drug), *Brimhana* (Anabolic), *Rasayana* (Enhance longevity &delays ageing) *and Vayahasthapana* (Age Prolonger) properties. Modified *Musta-Triphaladi Avaleha* is such a combination of all properties. *Avaleha* is a Good palatable drug for child.

Table 1: Ingredients of Modified *Musta-Triphaladi Avaleha*

No.	Drug Name	Latin Name	Part Used
1	*Musta*	*Cyprus rotundus* Nust.	Dry Rhizome
2	*Aamalaki*	*Emblica officinalis* Gaertn.	Dry Fruit
3	*Haritaki*	*Terminalia chebula* Retz.	Dry Fruit
4	*Vibhitaki*	*Terminalia bellerica* Roxb.	Dry Fruit
5	*Katuki*	*Picrorhiza kurroa* Royle ex Benth.	Dry Root
6	*Kakamachi*	*Solanum nigrum* Linn.	Dry Whole plant
7	*Kutaja*	*Holarrhena antidysenterica* Wall.	Dry Bark
8	*Haridra*	*Curcuma longa* Linn.	Dry Rhizome
9	*Vidanga*	*Embelia robusta* Burm	Dry Fruit
10	*Guduchi*	*Tinospora cordifolia* Willd.	Dry Stem
11	*Shweta Punarnava*	*Trianthema portulacastrum* Linn.	Dry Root
12	*Sharapunkha*	*Tephrosia purpurea* Linn.	Dry Root
13	*Apamarga*	*Achyranthus aspera* Linn.	Dry Whole plant
14	*Kadali*	*Musa paradisiacal* Linn,	Dry Rhizome
15	*Shatavari*	*Aspergus recemosus* Willd.	Dry Root
16	*Shigru*	*Moringa oleifera* Lam.	Dry Root bark
17	*Vasa*	*Adhatoda vasica* Nees	Dry Leaves
18	*Daruharidra*	*Berberis aristata* DC	Dry Root
19	*Sariva*	*Hemidesmus indicus* R.Br.	Dry Root
20	*Manjishtha*	*Rubia cordifolia* Linn.	Dry Root
21*	*Agnimantha*	*Clerodendrum Phlomidis.Linn.*	Dry Root
22*	*Rohitaka*	*Tecomella undulate* seem.	Dry Root
23*	*Agatsya*	*Sesbania grandifolia linn.*	Leaves
24*	*Kumari*	*Aloe barbadensis Mill.*	Leaves
25*	*Devadar*	*Cedrus deodara Roxb.*	Dry Bark

21	*Madhu*	Honey	----
22	*Sharkara*	*Saccharum officinarum* Linn	Crystal
23	*Chaturjata*		
	Twak	*Cinnamomum zeylanicum* Blume	Dry Bark
	Ela	*Elettaria cardamomum* Maton	Dry Seed
	Tamalapatra	*Cinnamomum tamala* Nees & Eberm	Dry Leaf
	Nagakesara	*Mesua ferrea* Linn	Dry Stamen
24	*Trikatu*		
	Shunthi	*Zingiber officinale* Rosc.	Dry Rhizome
	Maricha	*Piper nigrum* Linn.	Dry Fruit
	Pippali	*Piper longum* Linn.	Dry Fruit

*Drug were added in *Musta- Triphaladi Avaleha.*

The test drug was prepared in the Pharmacy, Gujarat Ayurved University, Jamnagar, by following Standard Operating Procedures (S.O.P.) of preparation of *Avaleha* as practiced in the pharmacy. *Avaleha* is palatable for Children. No any preservative was added to the test drug. Drug preparation was finished on 07.01.2017. Finished product was stored in airtight, sterilized food grade plastic containers at room temperature under hygenic condition. Modified *Musta Tiphaladi Avaleha* administerd in the Thalassemia major children. It was necessary to prepare the formulation in a better dosage form which is also free from microbial contamination, stability of a pharmaceutical product is the capability of a perticular formulation in a specific container or closure system, to remail within its physical, chamical, microbiological therapeutic specifications. Thus in the present study on attempt was taken to check stability of *avaleha* with respect to its Microbial profile at different climacteric conditions and temperature setups at regular interval for a period of 1 year.

Aim: To study the microbial contamination in the finished product at different time interval at different climacteric conditions, temperature and humidity set ups.

Materials and Methods: Sample; of Modified *Musta- Triphaladi Avaleha* were prepared (stored at room temperature) and studied to check microbial contamination at regular intervals for a period of one year. Microbiological study has been carried out in Microbiology Laboratory, of Institute. Mainly 02 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 on second day of preperation, Before issuing to the patients. Then samples from containers were subjected to the microbilogical study regularly with random intervals during different seasons.

Contents of Samples:

The sample contents 1 gm of Modified *Musta Triphaladi Avaleha*, 25 ingredients added with Honey & *Sarkara*, also added *Prakshepadravya* used specific proportion of all contents it was followed by fix dosage form of *Avaleha* which will easier to take for children. The whole plant of *Sharapunkha (Tephrosiapurpurea* Linn.*)* was purchased from the local market and rhizomes of *Kadali (Musa paradisiacal* Linn.*)* were collected from the behind of pharmacy, GAU, Jamnagar and *Agatsyapatra* collect from Kodinar, Gujarat. other remaining drugs were provided by the raw drug store of Pharmacy. *Agatsyapatra* collected from Kodinar district, Gujarat.

Preparation Time: The drug was prepared in the Pharmacy, Gujarat Ayurved University, Jamnagar, by following Standard Operating Procedures (S.O.P.) of preparation of *Avaleha* as practiced in the pharmacy with the utmost caution to avoid any sort of contamination.

Date of preparation: 07 January 2017

Storage: Finished product of Modified *Musta-Triphaladi Avaleha* was stored in air tight, sterilized food grade, plastic containers, smeared with ghee inside, stored in the open light area in the department at room temperature. Clean and dry stainless steel

spoon was used to take medicine. After emptying the container A, container B and C were used respectively in the same manner.

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:

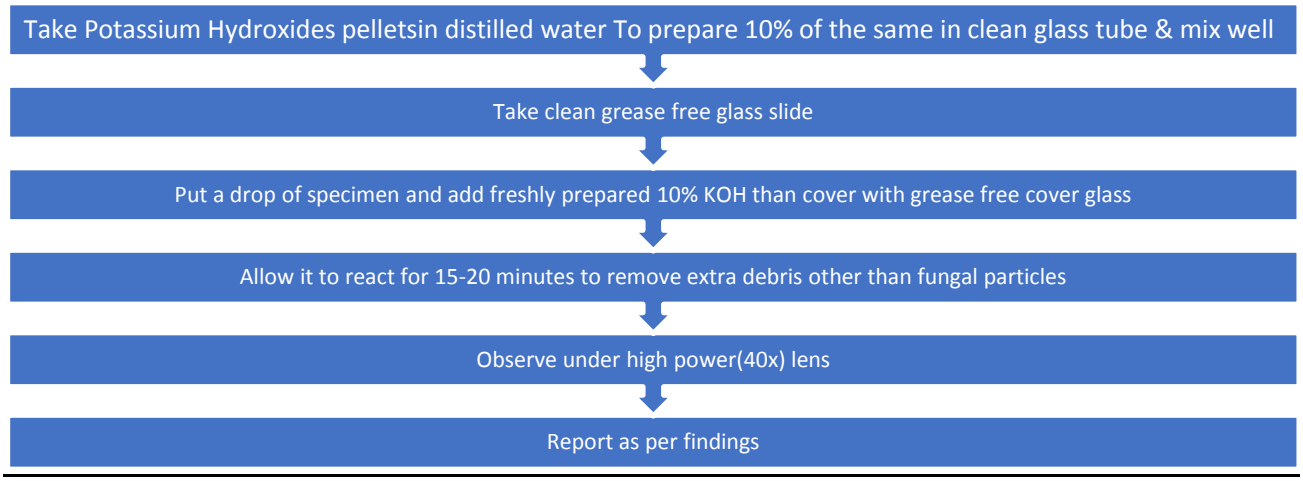
A. Wet mount /10% K.O.H. Preparation (Chart No. 1&2):

Aim: wet mount test for to rule out mycological findings in *Avaleha*

Specimen: As above mentioned

Chart 1: Procedure for Wet mount /10% K.O.H. Preparation

Chart 2: Procedure For 10% KOH Preparation

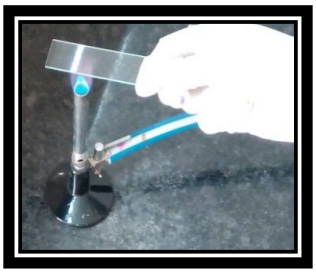


B. Gram's stain test (Chart No 3): Gram staining is a differential staining technique that differentiates bacteria into two groups: gram-positive and gram-negative. The procedure is based on the ability of microorganisms to retain color of the stains used during the gram stain procedure. Gram negative 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)[2]

Aim: Gram's stain is to rule out any bacteriological findings from *Avaleha*.

Specimen: As Mentioned above



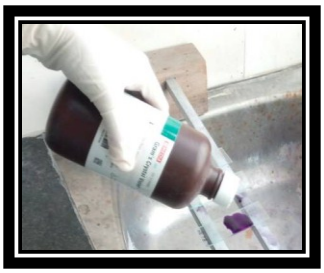


Figure 1 & 2: Smear staining Procedure

Chart 3: Procedure For Gram's Stain

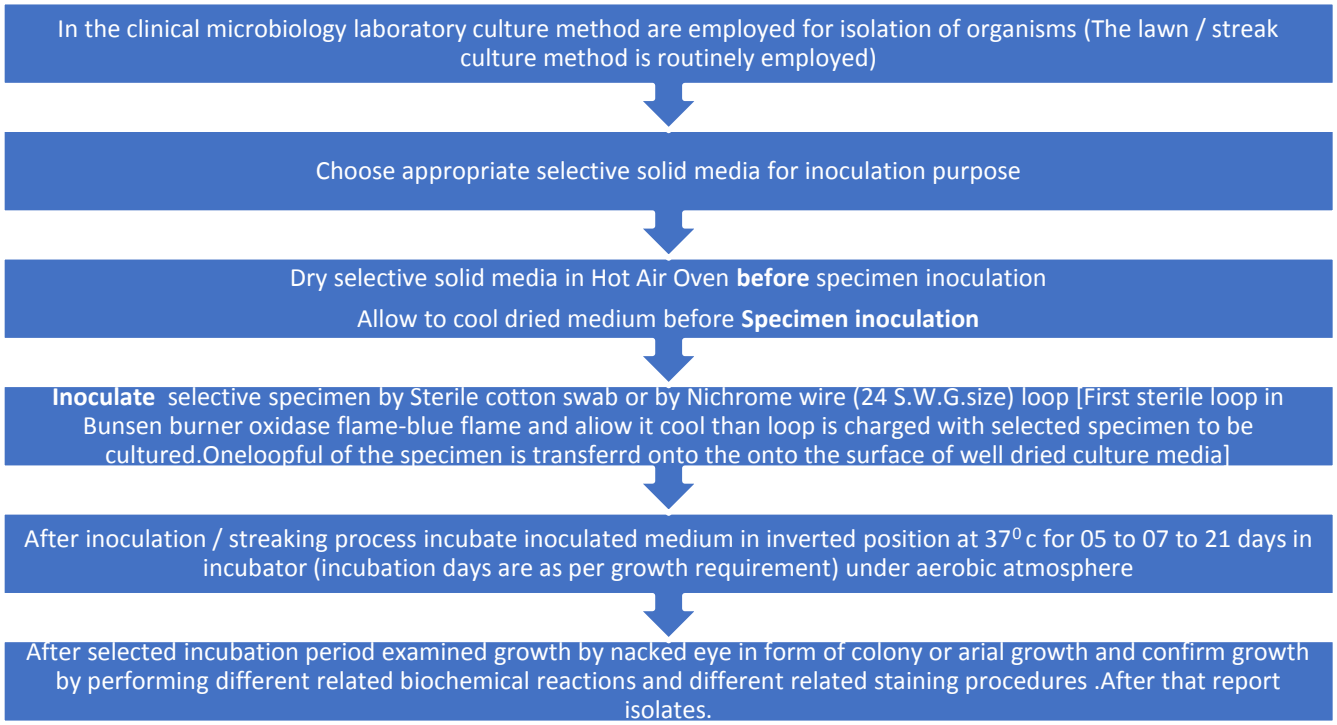
2. Culture Study

A Fungal culture method as given in Chart No. 4:

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.

Figure 3: Sabouraud Dextrose Agar Base (SDA) bottle

Chart 4: Procedure for Fungal Culture



B. Aerobic culture method as given in Chart No. 5:

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.

Figure 4: MacConkey Agar (MA)

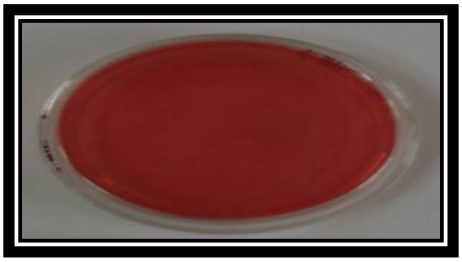


Chart No 5: Procedure for Aerobic Culture

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

Observations And Results 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.

Observation are shown in Table 1.

Table 1: Showing Observation of sample preserved at room temperature.

Days of investigations After preparation of the sample at	Date of Sample given	Temperature	Humidity	Observations of sample			
				Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
23 Days	22th Feb 2017	32◦c	32%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
53 Days	23th March 2017	36◦c	18%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
77 Days	27th April 2017	37◦c	73%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
106 Days	22th May 2017	38◦c	34%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
137 Days	23th June 2017	32◦c	75%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
167 Days	24th July 2017	27◦c	89%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
203 Days	29th August 2017	28◦c	92%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
230 Days	25th Sep 2017	32◦c	57%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
261 Days	26th Oct 2017	35◦c	26%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
288 Days	23st Nov 2017	23◦c Light rain	28%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
316 Days	21th Dec 2017	28◦cPartly sunny	20%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
377 Days	30rd Jan 2018	32◦c Suny	24%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated

DISCUSSION

The unscientific methods of collection, storage, transportation and congenial climatic conditions to allow raw materials for herbal drugs prone to fungal & bacterial infestations. The raw materials collected using unscientific methods commonly exposed to many pathogenic contaminants and are often deteriorated by pathogenic microorganisms during handling and storage[3]. Therefore, lack of regulation for herbal supplement presents potential health risk, largely their contamination chances with pathogenic. Present study was figured to observe the stability study of Modified *Musta Triphaladi Avaleha* with respect to Microbial Contamination of sample prepared and preserved in different climacteric and temperature conditions. Which has been used in Research work in IPGT & RA in 2017 shows a very good and promising result in Thalassemia. It is needed to keep at safe place will be helpful for safe usage for a long time. Hence primary 10% KOH/wet mount test was used; it detects fungal elements present but may not necessarily identify the species of the fungi. **Gram's method** of staining used to distinguish gram-positive bacteria and gram-negative bacteria. Aerobic & Fungal Culture was also performed in every sitting. It studied at regular interval of 1 month for 1 year. At the end of study, it was observed all three containers were not showed presence of any Microbes.

CONCLUSION

Stability is usually expressed in term of Serviceable life, which is the time period from when the product is produced until the time it is intended to be consumed or used. Hence Microbiological study of the Modified *Musta-triphaladi Avaleha* showed that the quality of *Avaleha*is in a standard condition. There were no growth found of microorganisms (Bacterial or fungal)till 30st Jan 2018 i.e. 01 year from the date of preparation, shows its good shelf life. It can help in future for maintaining its optimum quality and safety

and also provide guideline to the type of packaging and storage conditions.

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