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Stability Study of *Vata* (*Ficus bengalensis* Linn.) *Jata* (Aerial Roots) *Kwatha* (Decoction) with Respect to Baseline Microbial Diagnostic Modalities

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Vata Jata, Kwatha, Ficus Bengalensis. Stability, Decoction.

ABSTRACT: Background*: Kwatha*, one of the most popular *Kalpana* amongst five basic *Kalpana*, is widely used therapeutically as well as pharmaceutically. Although a clear description is not available in *Vedic* literature whereas a detailed description is available in all *Samhitas* regarding its preparation, the proportion of water to be added, reduction in the volume of liquid, etc. **Aims:** To check the stability of classical liquid dosage form, *Vata (Ficus Bengalensis* Linn.*) Jata* (aerial roots) *Kwatha* (decoction) with respect to its microbial profile. **Materials and Methods:** Sample of *Vata Jata Kwatha* was prepared and studied to check microbial contamination at regular intervals. **Results:** Six batches were subjected to the microbiological study from the date of the preparation to the date of the last microbiological study. No, any contaminations were found in the microbiological study in a minimum of one day and a maximum of 7 days after the preparation of the sample. **Discussion:** Hence the present study was carried out to observe the stability study of *Vata Jata Kwatha* with respect to Microbial Contamination of the sample prepared and preserved in different climatic and temperature conditions. Thus, a baseline Microbial profile was studied at a regular interval. At the end of the study, it was found that the shelf life of *Vata Jata Kwatha* was varied as per humidity and temperature. **Conclusion:** In the microbiological study of *Vata Jata Kwatha* shows its stability in a minimum of one day and a maximum of seven days after the preparation of the sample.

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INTRODUCTION

Svarasa (juice), *Kalka* (paste), *Kwatha* (decoction), *Hima* (cold infusion) and *Phanta* (hot infusion) are considered as primary dosage forms in *Bheshaja Kalpana* (dosage forms). *Kwatha Kalpana* may be defined as a *Kalpana* in which a specific quantity of *Kwatha Dravya* (coarse powder)is taken, the specific amount of water is added, reduced overheat and filtered to obtain *Kwatha*. It is prepared by boiling the finely powdered plant material in the necessary quantity of water until all the active ingredients are extracted entirely into the water. The retained liquid after boiling is then filtered through a muslin cloth. The filtrate so obtained is known as '*Kwaatha'*- the decoction. According to '*Saarngadha Samhita* - a fixed quantity of sixteen parts of water in proportion to powdered plant material needs to be added and the eighth part

retained after boiling when the decoction is to be used for consumption [1]. The reason for boiling the plant material in water is to extract the entire water-soluble ingredients. The quantity of water required to be added and to be retained after boiling varies from plant to plant [2]. According to some experts whatever quantity of water is added, the three-fourth part of water needs to be evaporated and the fourth part retained to achieve this objective [3].

Vata (*Ficus bengalensis* Linn.) is a medicinal plant and dedicated to a religious purpose since Vedic times. An aqueous extract of the aerial roots along with salt is used in diabetes [4]. The whole plant is astringent, refrigerant, anodyne, vulnerary, depurative, anti-inflammatory ophthalmic, styptic, antiarthritic, diaphoretic, anti-diarrheal, antiemetic, tonic and possess pharmacological activities hypoglycemic, hypotensive, antifertility, antidiabetic, antioxidant, hypocholesterolemic. The aerial roots are useful in obstinate vomiting, leucorrhoea, and osteomalacia of the limb [5]. *Vata* (*Ficus benghalensis* Linn.) *Jata* (aerial roots) *Kwatha* (decoction) is used in various *Kalpa* (formulation) preparation like *Abhraka Bhasma* (incinerated Mica) [6], *Svarna Sindhura* (herbomineral formulation) [7], *Trivanga Bhasma* (incinerated Tin, Lead, Zinc) [8]. Review of literature reveals that few works have been reported on *Ficus benghalensis* Linn. which includes a review on ethnobotanical claims, chemical constituents, pharmacology biological activities [9-11], and characterization of fungal antagonistic bacilli isolated from aerial roots [12]. In Ayurveda, it is discussed under *Saviryata avadhi* that is the time period during which the potency *(Virya)* of a drug remains unaffected due to environmental or microbial factors [13]. A number of dosage forms are explained in Ayurveda, which have different shelf life, depends on various physical, chemical, environmental and biological factors. A few *Kalpanas* have very short life, such as *Swarasa* (juice), *Kalka* (paste), *Kwatha* (decoction), etc., while a few have longer shelf life like *Asava* (self-generated alcohol), *Bhasma* (calcined metals/minerals), etc [14]. Average shelf life of formulations under different categories has been specified through the gazettes of Government of India and it is an essential component to be displayed in the labelling. The amendment of Rule No. 161-B of Drugs and Cosmetic Act 1940, specify the maximum shelf life or date of expiry. The shelf life of fresh prepared *Kwatha* is not mentioned in the Gazette of India [15]. Shelf life of *Kwatha* as per *Yogaratnakara* is 01 *Prahar* (3hrs) but the available information on shelf life is general, it becomes essential to evaluate shelf life of individual formulations. Hence, the present topic of the stability test of *Vata Jata Kwatha* with respect to microbiological findings was selected in the study. The drug was prepared in RS and BK dept. I.P.G.T & R.A., Jamnagar of Gujarat Ayurved University, Jamnagar. No, any preservative was added to the test drug.

MATERIAL AND METHODS

Sample of coarse powder of *Vata Jata Kwatha* was prepared (stored at room temperature) and studied to check microbial contamination at regular intervals. The microbiological study has been carried out in Microbiology Laboratory, I. P. G. T. & R. A., Jamnagar.

Drug preparation:

Vata Jata Kwatha was prepared according to "Sharangadhara Samhita" [16]. *Vata Jata* was taken and soaked in water for overnight. The next day, it was subjected to mild heat with continuous stirring without covering its mouth. The reduction was done until the quantity became approximately $1/8^{th}$ of the original volume. Then *Kwatha* was filtered through double-folded clean cotton cloth. This filtered *Kwatha* was collected as *Vata Jata Kwatha*.

Drug material

The *Vata Jata Kwatha* was prepared in RS and BK dept. I.P.G.T & R.A, Jamnagar of Gujarat Ayurved University, Jamnagar.

Storage

The finished product was stored in air-tight food-grade, plastic containers, stored in the open light area in the department at room temperature. A clean and dry stainless-steel spoon was used to take medicine.

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: *Vata Jata Kwatha*
Procedure Potassium Hydroxide pellets were added in distilled water to prepare a 10% solution in a clean glass tube and it was mixed well. A clean grease-free glass slide was taken. Then a drop of the sample was put on it and freshly prepared 10% Potassium hydroxide (KOH) was added to it and after that sample was covered with a grease-free cover glass. Then it was allowed to react for 15-20 min to remove extra debris other than fungus. After that, the cover glass was observed under high power (40X) lens and the findings were noted down properly.

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 the 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 the decolorization step, a counterstain effect found on Gram-negative bacteria and bacteria will remain pink. The Gram stain procedure enables bacteria to retain the color of the stains, based on the differences in the chemical and physical properties of the cell wall (Alfred E Brown, 2001) [17,18]

To rule out any bacteriological findings following the procedure adopted

Specimen: *Vata Jata Kwatha*

Procedure for Gram's Stain [**Figures 1-3**] The clean grease-free glass slide was taken to prepare dry equal thick preparation i.e. smear. Then the smear was fixed by passing 3-4 times over the flame of a Bunsen burner. This fixation kills the vegetative form of microbes, render them permeable to stain and make the material stick to the surface of the slide and prevent autolytic changes. Fixed prepared smear was covered with Gram's crystal violet solution and it was allowed to remain for mentioning time as per the kit procedure. Then the smear was washed off with tap water to remove excessive reagent. Then smear was covered with Gram's Iodine solution and it was allowed to remain for some time. Again, the smear was washed off with tap water to remove excessive reagent. The smear was decolorized with Gram's de-colorizer by holding the slide at a slant position and pour Gram's decolorized acetone from its upper end up to the removal of the color of primary dye i.e Gram's Crystal Violet. Then the smear was washed off with tap water to remove excessive reagent. After that, the smear was covered with Safranin solution and it was allowed to remain for mentioning time as per the kit procedure. Then the smear was again washed off with tap water to remove excessive reagent. The smear was blotted and it was allowed to dry. Then the slide was examined under the oil immersion lens and the findings were properly noted.

Fig. 1

Fig. 2

Fig-1 & 2 - Smear Staining Procedure

A. Fungal culture method:

Respected materials collected with a 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.

Fig-3- Sabouraud Dextrose Agar Base (SDA) Bottle

Procedure for Fungal Culture In the clinical microbiology laboratory, the culture method was employed in the isolation of organisms (The streak culture method was routinely employed). Then appropriate selective solid media i. e Sabouraud Dextrose Agar Base (SDA), Modified (Dextrose Agar Base, Emmons) for inoculation purpose was selected. After that, the selective solid media were dried in Hot air oven and the dried medium was allowed to cool before specimen inoculation. Selective samples were inoculated by sterile cotton swab to the surface of well-dried culture media. After the streaking process inoculated medium was incubated in an inverted position at 37 ° C for 5-7 days. After incubation period growth was examined by the naked eye in the form of the colony and the growth was confirmed by performing different related biochemical reactions and different staining procedures. After that results were noted down properly

Aerobic culture method:

Respected materials collected with a 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 ºC

Use of media: for selective cultivation of pathogenic bacteria.

Procedure for Aerobic Culture in the clinical microbiology laboratory, the culture method was employed in the isolation of an organism (The streak culture method was routinely employed). Appropriate solid media, i.e. MacConkey Agar (MA) and Columbia Blood agar (BA) were selected for inoculation purposes. Then selective solid media were dried in a hot air oven and the dried medium was allowed to cool before specimen inoculation. Then the selected sample was inoculated on the surface of the cool dried medium with a sterile cotton swab to the surface of well-dried culture media. After the streaking process inoculated medium was incubated in an inverted position at 37 °C for 24-48 h in the incubator under the aerobic condition and 10% Carbon di-oxide (CO2) atmospheric condition. After selecting the incubation period,

growth was examined by the naked eye in the form of colonization, and growth was confirmed by performing different related biochemical reactions and different related staining procedures. After that, the reports were isolated and noted down properly.

RESULTS AND DISCUSSION

Organoleptic evaluation

The aerial root decoction was dark brown with a characteristic slightly aromatic odor, astringent in taste and smooth liquid in touch and are mentioned in **Table no 4**.

Specific gravity Total solid content and, **pH of *Vata Jata Kwatha***

The average of specific gravity, total solid content and, pH of *Vata Jata Kwatha* were 1.131,2.20 and 8.21respectively. [mentioned in **Table no 4**]

Microbiological Study

Every time sample (in which drug preserved) were subjected to the microbiological study from the date of the preparation to the date of the last microbiological study. This was a very preliminary study. Six out of six samples showed growth at various periods. In batch one, the smear shows the presence of many capsulated gram-negative rods arranged singly, and Escherichia coli was isolated on the 2nd day after preparation. Results were shown in **Table no 1**.

The present study was carried out to observe the stability of *Vata Jata Kwatha* with respect to microbial contamination of samples prepared and preserved in different climatic and temperature conditions. Thus, a baseline microbial profile was studied at a regular interval. At the end of the study, it was found that sample was showed the presence of microbes in batch one on 2nd day from date of preparation, in batch two on 1st day from the date preparation, in batch three and four on 3rd day, in batch five on 7th day and in batch six on 3rd day from date of preparation were found. The main factors affecting the Shelf life are a derivation of the drug, dosage forms, environmental factors (humidity, temperature, light), microbial contamination, storage conditions & packaging system, etc [19. Stability is usually expressed in terms of shelf-life, which is the period from when the product is produced until the time it is intended to be consumed or used.

Table 1: Showing observations of *Vata Jata Kwatha* preserved at room temperature

Sr. No.	Days of investigations After preparation of the sample at	Date of Sample given	Observations of sample			
			Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
Batch -1						
1.	Day-1	18/7/2018	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
	Day-2	19/7/2018	The smear shows the presence of many capsulated gram-negative rods arranged singly	Escherichia coli isolated	Fungal filaments not seen	No Fungal Pathogen Isolate
Batch -2						
2.	Day-1	19/7/2018	The smear shows the presence of many capsulated gram-negative rods arranged singly	Escherichia coli isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
Batch -3						
3.	Day-1	23/8/2018	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
4.	Day-2	24/8/ 2018	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
	Day-3		The smear shows the presence of many capsulated gram-negative rods arranged singly	Escherichia coli isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated

Batch -4						
5.	Day-1	27/8/2018	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
	Day-2	28/8/2018	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	Fungal filaments not seen.
	Day-3	29/8/2018	The smear shows the presence of many capsulated gram-negative rods arranged singly	Escherichia coli isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
Batch -5						
	Day-1	24/04/19	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
	Day-2	25/04/19	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
	Day-3	26/04/19	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
	Day-4	27/04/19	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
	Day-5	28/04/19	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
	Day-6	29/04/19	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
	Day-7	30/04/19	Microorganisms Not Seen	No organisms isolated	A structure resembling filamentous fungus (Dimorphic yeast) seen.	Candida albicans isolated after 04 days of incubation at 37^0C under Aerobic Atmosphere
Batch -6						
	Day-1	1/ 07/2019	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
	Day-2	2/ 07/2019	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
	Day-3	3/ 07/2019	The smear shows the presence of many capsulated gram-negative rods arranged singly	Escherichia coli isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated

Temperature and humidity chart

Table 2: Showing observations of *Vata Jata Kwatha* preserved at room temperature:

Days of investigations After preparation of the sample at	Date of Sample given	Observations of sample			
		Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
Batch 1					
Day-1	18 /07/2018	26°C	100%	Fungal filaments not seen.	No Fungal Pathogen Isolated
Day-2	19 /07/2018	30 °C	77%	Fungal filaments not seen.	No Fungal Pathogen Isolated
Batch 2					
Day-2	19 /07/2018	30 °C	77%	Fungal filaments not seen.	No Fungal Pathogen Isolated
Batch -3					
Day-1	23/8/2018	28 °C	80%	Fungal filaments not seen.	No Fungal Pathogen Isolated
Day-2	24/8/ 2018	28 °C	78%	Fungal filaments not seen.	No Fungal Pathogen Isolated
Day-3	25/8/ 2018	26 °C	89%	Fungal filaments not seen.	No Fungal Pathogen Isolated
Batch -4					
Day-1	27/8/2018	27 °C	77%	Fungal filaments not seen.	No Fungal Pathogen Isolated
Day-2	28/8/2018	29 °C	76%	Fungal filaments not seen.	No Fungal Pathogen Isolated
Day-3	28/8/2018	27 °C	81%	Fungal filaments not seen.	No Fungal Pathogen Isolated
Batch -5					
Day-1	24/04/19	35 °C	29%	Fungal filaments not seen.	No Fungal Pathogen Isolated
Day-2	25/04/19	35 °C	32%	Fungal filaments not seen.	No Fungal Pathogen Isolated
Day-3	26/04/19	37 °C	26%	Fungal filaments not seen.	No Fungal Pathogen Isolated
Day-4	27/04/19	41 °C	15%	Fungal filaments not seen.	No Fungal Pathogen Isolated
Day-5	28/04/19	39 °C	17%	Fungal filaments not seen.	No Fungal Pathogen Isolated
Day-6	29/04/19	39 °C	20%	Fungal filaments not seen.	No Fungal Pathogen Isolated
Day-7	30/04/19	37 °C	16%	A structure resembling filamentous fungus (Dimorphic yeast) seen.	Candida albicans isolated after 04 days of incubation at 37^0C under Aerobic Atmosphere
Batch -6					
Day-1	1/ 07/2019	32 °C	59%	Fungal filaments not seen.	No Fungal Pathogen Isolated
Day-2	2/ 07/2019	33 °C	57%	Fungal filaments not seen.	No Fungal Pathogen Isolated
Day-3	3/ 07/2019	33 °C	59%	Fungal filaments not seen.	No Fungal Pathogen Isolated

Table 3: Specific gravity Total solid content and, pH of *Vata Jata Kwatha*

	Specific gravity	Total Solid Content	pH
Batch 1	1.1298	1.75	7.80
Batch 2	1.1306	2.23	8.1
Batch 3	1.1310	2.26	8.20
Batch 4	1.1335	2.12	8.4
Batch 5	1.130	1.22	8.2
Batch 6	1.1336	1.77	8.6
Mean	1.131	2.20	8.21

Table 4: Organoleptic characteristics of *Vata Jata Kwatha*

Parameters	*Vata Jata Kwatha*
Sparsa (consistency)	Smooth liquid
Rupa (Colour)	Dark Brown
Rasa (Taste)	Astringent *(Kashaya)*
Gandha (Odour)	Characteristic slightly aromatic

Microorganism needs water, humidity, and temperature at suitable environmental conditions to develop in any media, surface, or article. Shelf life according to *Yogaratnakara* is 01 *Prahar* (3hrs) [15]. In batch two, it was found that contamination on the first day from the date of preparation which was the same as acharya *Yogaratnakara* opinion but in batch three, four and six, contamination was found on the third day. From the above finding, it revealed that the shelf life of *Vata Jata Kwatha* was varied as per humidity and temperature. (**Table 1 and Table 2**). One of the classical liquid dosage forms like *Kumari* (*Aloe vera.* [L.] burm.)*) Swarasa* (juice) shows its stability in the range of a minimum of one day and a maximum of three days after the preparation of the sample [20] whereas *Vata Jata Kwatha* shows its stability in the range of a minimum one day and a maximum of seven days after preparation of the sample. Due to the boiling procedure in the preparation, *Kwatha* may have prolonged the range as compare to *Aloe vera* juice.

CONCLUSION

The microbiological study of *Vata Jata Kwatha* shows its stability in the range of a minimum one day and a maximum of seven days after the preparation of the sample.

DATA AVAILABILITY STATEMENT

Not declared.

ETHICS STATEMENT

The authors have taken all the necessary permissions as per ethical guidelines wherever applicable. The authors will be responsible for all the technical content mentioned in the manuscript. Journal and Publisher will not be responsible for any copyright infringement and plagiarism issue.

AUTHOR CONTRIBUTIONS

The entire study was conceptualized, designed and conducted the study with the help of other authors, wrote the first draft of the manuscript, and other authors contributed significantly to the revision of the manuscript. All Authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Declared none.

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