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Pharmaceutical analytical study of *Baladi Kwatha* and to evaluate the effect of various concentration of preservatives in it

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ABSTRACT

Due to advent of commercialization longer shelf-life becomes the need of the hour for various pharmaceutical products, especially for the preparation of *Kwatha Kalpana* (Decoction). The shelf-life of *Kwatha* is 1 *Prahara* (3 hours). According to *Acharya Sharangadhara* it is considered as *Sadhyo-sevana. Panchavidha Kashaya Kalpana* are considered as basic pharmaceutical preparation and the most important form of *Kalpana.* But the preparation of *Kwatha* is not possible all the times as the shelf life of *Kwatha* is short and makes it impossible to market it as soon as it is prepared. Therefore, there is need to find different ways to preserve the *Kwatha* for longer duration for their easier usage and transportation. This present study is carried out mainly in 3 stages, that is, pharmaceutical study, analytical study and microbiological study. In pharmaceutical study, the preparation of *Baladi Kwatha* was carried out as per the classical reference of *Shamana Kashaya* and they were added with different ratios of preservatives (SB-0.1%, SB-0.2%, SB-0.2% + MP-0.05% + PP-0.05%, SB-0.48% + MP-0.1% + PP-0.1%). No much changes were noted in analytical studies. Preservatives like, sodium benzoate, in permitted amounts could only preserve acidic *Kwatha* for a shorter period. Hence, marketing *Baladi Kwatha* with sodium benzoate cannot be properly stabilized by 0.1% & was observed that at least 0.2% is required to preserve the *Kwatha* for 60 days free from microbial contamination. It was observed that as per the permissible limit, the preservatives when used in combination of sodium benzoate 0.2%, methyl paraben 0.05%, propyl paraben 0.05%, has passed the shelf-life for 3 months.

Key words: *Baladi Kwatha, Panchavidha Kashaya Kalpana, Shelf life, Preservatives.*

INTRODUCTION

Vedic period is considered as the first period of documentation of references regarding the use of herbal drugs. *Acharya Charaka* has coined the term "*Kalpana*" for the pharmaceutical preparations.[1] The raw drugs will be converted into different dosage forms by considering *Roga Bala, Rogi Bala*[2] etc. Among this, few formulations are prepared instantaneously which cannot be preserved for longer duration, with some exceptions. In ancient period, our *Acharyas/ Vaidyas* used to prepare medicines by themselves in small scale to treat their patents, here the main objective was to achieve desired action rather than palatability/ shelf-life. But in today's era of globalization & industrialization, leading to large scale production there is a need to determine the stability period of these *Kalpanas* by the guidance of certain rules mentioned in *Samhitas* and books. In the present era, most abundantly used *Kalpana* among *Panchavidha Kashaya Kalpana* is *Kwatha Kalpana.*[3] It is used for various conditions but the shelf life of *Kwatha* is less, therefore this *Kwatha* is preserved in different ways by the manufacturers. Ex: *Kwatha* tablets, *Ghanavati, Arishta* etc. The *Kalpana* with short

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span of shelf-life is affected from various hazards like microbial contamination which lead to degradation of products. Pharmaceutical dosage forms contain carbohydrates, lipids, proteins, steroids, inorganic salts, minerals, water etc besides active drug components in it. These ingredients might serve as a nutrient for growth of various microorganisms. Government has approved to add food grade preservatives for ayurvedic formulations. But the quantity, compatibility of these preservatives varies and differs according to the formulation. Also, different formulations will have different shelf-life based on ingredients present in their process of preparation, moisture content etc. *Panchavidha Kashaya Kalpana* are primary pharmaceutical preparation and the most important form of *Kalpana*. Regarding the shelf-life of *Kwatha, Acharya Yogaratnakara* explains it as 1 *Prahara*[4] (3hrs) & *Acharya Sharangadhara* explains it as *Sadhyosevana.*[5] Due to very short shelf-life of *Kwatha* it makes inappropriate to market it to patients in current scenario & also creates inconvenience in day-to-day practice. Aqueous preparations like *Kashaya, Swarasa*, etc. are least resistant against microorganisms, here the deterioration is mainly due to microbial growth. To prevent this microbial growth and stabilize the formulation, stabilizers are added to it. Selection of preservatives depends on the formulation and also the presence of aqueous media. This study is an attempt made to understand the appropriate use of preservatives in *Baladi Kwatha*[6] which is prepared as per the classical method of *Kwatha Kalpana.*[7] Preservatives used in this study are Sodium Benzoate, Methyl Paraben and Propyl Paraben. According to this study, the samples which pass the stability with minimum ratio of preservatives can be used for ensuring the longer shelf life of *Baladi Kwatha*.

Materials and Methods

Collection of raw drugs

The raw drugs were collected from Kajrekar Pharmacy, Belagum after identifying the *Grahya Lakshanas* mentioned in the classics. Every raw drug was identified and authenticated from *Dravyaguna* Department for their genuinity. The chemical preservatives: Sodium benzoate, methyl paraben & propyl paraben were bought from genuine chemicals dealer Vasa Scientifics, Bengaluru.

Processing of raw drugs

All the raw drugs were checked for physical impurities and subjected to washing and drying.

Method of preparation of *Baladi Kwatha*

Equipment's: Pulverizer, weighing machine, vessel, stirrer, cloth, gas stove, pyrometer, glass bottle.

Table 1: Ingredients of *Baladi Kwatha.*

SN	Ingredients	Parts used	Quantity
1.	*Sida cordifolia*	Root	700 grams
2.	*Sida rhombifolia*	Root	700 grams
3.	*Zingiber officinale*	Rhizome	700 grams
4.	Water	-	16.8 litres

The raw drugs were taken in pulverizer of mesh size 40 for obtaining coarse powder, which were later weighed and utilized for the preparation of *Kwatha* in a copper vessel. To 1 part of coarse powder of drug, 16 parts of water was added and kept over fire. The temperature of *Kwatha* during boiling was maintained in between 80°C – 90°C & outside 22°C- 28°C. It was then filtered using a thick white kora cloth and allowed for self-cooling. Later it was filled in glass container. Preservatives in different ratio are added to it & stored at room temperature.

Here, the colour of the *Kwatha* changed from light brown to dark brown colour. The consistency of the liquid was gradually increased. The smell of the raw drugs was appreciated, especially the smell of *Shunti*. The atmospheric temperature noted on the day of preparation was 22°C – 28°C and humidity was 58%.

Table 2: Final yield of *Baladi Kwatha*

Parameters	Batch A
Kashaya Choorna quantity	2.1kg
Total quantity of water	16.8litres
Temperature given	80°C – 90°C
Time taken for reduction	5hrs 30mins

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Total quantity of *Kashaya* obtained	2.1 litres

Final yield of *Baladi Kwatha*: 2.1 litres

Packing and dose schedule of the preservatives

The prepared *Baladi Kwatha* was filled into narrow mouthed sealed glass bottles which were previously sterilized and preservatives in various concentration were added to *Baladi Kwatha*. After adding the preservatives, the contents in the bottle were shaken well to ensure proper mixing. Then these bottles were tightly sealed and left at the room temperature for entire course of the study. Temperature in Bengaluru was noted between 22°C – 28°C and humidity was 58%. 50ml of each sample of *Kwatha* was taken for the study every month. Freshly prepared *Baladi Kwatha* & the *Kwatha* with added preservatives were used for both analytical & microbiological studies. The sterility of the *Kwatha* was tested after adding the preservatives for microbial load at the interval of one month for 6 consecutive months. A *Kwatha* sample without adding preservatives served as control.

Observation and Results

Table 3: Preparation of samples[8]

Without preservatives	Sample A
With adding preservatives 0.1%	Sample B
With adding preservatives 0.2%	Sample C
With adding preservatives- sodium benzoate 0.2%, methyl paraben 0.05%, propyl paraben 0.05%	Sample D
With adding preservatives- sodium benzoate 0.48%, methyl paraben 0.1%, propyl paraben 0.1%	Sample E

Table 4: pH of *Baladi Kwatha* without adding preservatives.

Time duration	pH
0hr	4.12
3hr	4.16
12hr	4.60
24hr	4.90

Table 5: pH of *Baladi Kwatha* with preservatives.

No of Samples	Instant	30 days	60 days	90 days	120 days
Sample B	4.12	4.6	5.94	6.53	7.9
Sample C	4.16	4.3	4.96	6.12	7.8
Sample D	4.2	4.36	4.58	5.96	6.91
Sample E	4.23	4.27	4.38	5.78	6.97

Table 6: Refractive index of *Baladi Kwatha* with & without preservatives.

No of samples	Instant	30 days	60 days	90 days	120 days
Sample A	1.35408	0	0	0	0
Sample B	0	1.34578	1.34608	1.35012	1.36035
Sample C	0	1.35112	1.35324	1.35216	1.35224
Sample D	0	1.3525	1.34464	1.35433	1.35672
Sample E	0	1.34987	1.3569	1.35476	1.35641

Table 7: Specific gravity of *Baladi Kwatha* with & without preservatives.

No of Samples	Instant	30 days	60 days	90 days	120 days
Sample A	1.0422	0	0	0	0
Sample B	0	1.0502	1.0622	1.0655	1.0632
Sample C	0	1.0567	1.0590	1.0537	1.0593

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Sample D	0	1.0542	1.0521	1.0597	1.0618
Sample E	0	1.0532	1.059	1.0579	1.0633

Table 8: Total solids of *Kwatha* with & without preservatives.

No of samples	Instant	30 days	60 days	90 days	120 days
Sample A	11.52	0	0	0	0
Sample B	0	11.45	11.49	11.23	11.57
Sample C	0	11.71	11.75	11.78	11.49
Sample D	0	11.53	11.59	12.15	11.87
Sample E	0	11.64	11.66	11.71	11.79

Preparation of Casein Soya Bean Digest Agar Medium (CSDAM).

Materials required for the preparation of agar medium: Weighing machine, Petri dish, Jar, Stirrer, Spatula, pH meter

Ingredients required:

Chemicals	Quantity
Peptone	15gms
Soya peptone	5gms
Sodium chloride	5gms
Distilled water	990ml
Agar	15gms

Dissolve peptone (15g), soya peptone (5g), sodium chloride (5g) in 990ml of distilled water. pH of this was adjusted to 7.3 using digital pH meter and make up the volume to 1000ml. finally we have to add 15g of agar to the media and autoclaved at 121°C for 20 mins.

Total aerobic microbial count by plate count method:

Materials required: Weighing balance, Petri dish, Jar, Stirrer, Spatula, Laminar air flow, Loop, Single burner, Autoclave, Incubator, Micro titre pipette.

Procedure: Clean the working place in laminar airflow using 70% ethanol and switch on the UV for 20 mins. After cooling, 15ml of casein soya bean agar media of 45°C was poured into 100mm petri dishes. 1ml of undiluted *Baladi Kwatha* was added into the petri dish containing the media. Plates were gently rotated in a circular motion to achieve uniform distribution of the sample and the media was allowed to solidify. All the petri dishes were incubated for 5 days at 28°C in BOD incubator. All the experiment was carried out in triplicate. Number of colonies were counted using digital colony counter. One batch was taken for microbial load and conducted by same person. 50ml of each of the undiluted *Baladi Kwatha* sample were used to the study. Freshly prepared *Kwatha* and *Kwatha* with preservatives, both were used. The sterility of the *Kwatha* was tested after adding the preservatives for microbial load at the intervals as explained in the table.

Results of Microbial Load

The above study shows that the freshly prepared *Baladi Kwatha* had no microbial growth when tested at 0hr but when tested for 3hr and 12 hr it showed slight growth.

The above study shows that the sample C and sample E were found to be equally effective after 30 days of the study. There was no significant microbial growth in all the samples to consider it as spoilt.

There was a significant microbial growth in sample B which was considered as spoilt on 60th day. All the other samples were not considered as spoilt. Sample C and Sample E were found to be equally effective.

There was significant microbial growth in sample C and it was considered as spoilt. But the sample D had microbial growth but it was under permissible limit so it was not considered as spoilt. Sample E did not have any microbial growth.

There was a significant microbial growth in sample D and E so it was considered as spoilt.

The result of this study shows that the antimicrobial activity of the preservatives is more when used in combination with same preservatives than when used individually, a finding that contributes to be more

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effective & safer. Sodium benzoate alone when used in higher ratio showed near to equal effect to the sample which had combination of preservatives.

Discussion

The *Kwatha* was prepared by adding 8 parts of water to 1 part of drug. The reduction was carried out 1/8th (*Shamana Kashaya*). Microbial load of all the samples of *Baladi Kwatha* was carried out at different intervals of time. The samples were clean without the microbial growth for 90 days. On 120th day it was found that the samples had crossed the limit and was not taken for further studies. As per the studies the sample C (SB-0.2%) and sample E (SB-0.48% + MP-0.1% + PP-0.1%) showed somewhat equal results after 30 days of preservation. Sample B (SB-0.1%) showed shelf life for 30 days, Sample C (SB-0.2%) showed for 60 days, But the sample D (SB-0.2% + MP-0.05% + PP-0.05%) and E (SB-0.48% + MP-0.1% + PP-0.1%) both were stable for 90 days after which both the samples were spoilt. The chemical constituents such as essential oils, pungent phenolic compounds, gingerols may help in preservation. Because of this, there is way and the method of bio preservatives to evaluate their efficacy in extending the shelf life and improving the microbial safety of pharmaceutical products. By this study, it was found that the *Baladi Kwatha* was better preserved when the preservatives were used in combination. It may be due to the synergic action of this combination with the chemical constituent present in it and the acidic pH of *Baladi Kwatha*. The reduction of *Kwatha* also plays an important role in the preservation, here 1/8th reduction was carried out but 1/16th reduction might have increased the shelf life of *Baladi Kwatha* as the water content would be less. In this study all the samples were stored in colourless glass bottle & and the bottles once opened were not used for next studies as it will have contact with the atmosphere making it vulnerable for contamination. Therefore, each time separate bottles were used for testing of microbial load. It has been observed that the taste of both sodium benzoate and methyl paraben was sweet and the propyl benzoate was slightly sweet. As the glass bottle is inert, the *Kwatha* should be preferably stored in this.

Conclusion

Preservatives as sodium benzoate in permitted amounts could only preserve acidic *Kwatha* for a shorter period, hence marketing *Baladi Kwatha* with sodium benzoate cannot be properly stabilized by 0.1% & was observed that at least 0.2% is required to preserve the *Kwatha* for 60 days free from microbial contamination. It was observed that as per the permissible limit, the preservatives when used in combination sodium benzoate 0.2%, methyl paraben 0.05% propyl paraben 0.05%, has passed the shelf-life for 3 months when the reduction of *Kwatha* was 1/8th.

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