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Standard manufacturing procedure and quality parameters of *Kanakabindvarishta*

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Abstract

Kanakabindvarishta is a Ayurvedic formulation described in *Charaka Samhita* in the context of *kushtarog* (Skin disorders). In the reference, the ratio to prepare the *Kwatha* of *Khadira* and which sweetening agent should be used is not mentioned. So efforts have been carried out to decide that and to generate SMP of *Kanakabindvarishta*. In present study, total 5 batches of *Kanakabindvarishta* were prepared by adding yeast. The heartwood of *Khadira* was procured from the forest of Saurashtra Region. Organoleptic and Physico- chemical analysis like LOD, pH value, water and methanol soluble extractive, sugar and alcohol contents and Qualitative test for various functional groups were carried out. Fermentation started and ceased earlier because of addition of the yeast. The yield was found in between 58.92 to 68.60 %. Very less variation found in different parameters in all batches. Tannin percentage of *Khadira* and final product was 7.1 and 4.09% respectively that shows utilization of tannin in fermentation process. By deciding Ratio of *kwatha* and Sweetening agent according to description of *acharya Sharangdhar*, the S.M.P has been developed to prepare *Kanakabindvarishta*.

Keywords: Acacia catechu, *Kanakabindvarishta*, *Khadira*, Quality Parameters, S.M.P.

1. Introduction

Asavas and *aristas* are medicinal preparations made by soaking the drugs, either in coarse powder form or in the form of *kashaya* (decoction), in a solution of sugar or jaggery, as the case may be, for a specified period of time, during which it undergoes a process of fermentation generating alcohol, thus facilitating the extraction of the active principles contained in the drugs [1]. These formulations have longer shelf life, quick absorption and action and excellent therapeutic efficacy as compared to other Ayurvedic herbal medicines [2]. Pharmaceutically *Asava arista* take about a month to prepare. But, at Industrial level, general trends are going on to use some kind of fermenting agent (i.e. yeast) for the early initiation and cessation of the fermentation process to reduce its preparation time for manufacturing benefits. One study on the effect of addition of yeast reveals that the onset and completion of fermentation process in the samples containing yeast were quick in compare to the sample containing *Dhatiki Pushpa* (Flower of *Woodfordia fruticosa* Kurz.) [3]. Here Yeast has been taken as a fermenting agent to prepare *Kanakabindvarishta*. *Kanakabindvarishta* is a well-known Ayurvedic formulation firstly described in *Charaka Samhita* [4]. *Khadira* (*Acacia catechu* Willd.) is a main drug of *Kanakabindvarishta* along with having several potent drugs which play a major role in the specific pharmaco-dynamic and pharmacokinetic actions of the formulation. Thus in this study a trial has been made to generate SMP and quality control parameters of *Kanakabindvarishta* at pharmaceutical, physico-chemical and phyto-chemical level.

2. Materials and Methods

Five batches were prepared as per reference in *Charaka Samhita* with modification of adding yeast. (Table 1)

2.1 Procurement and preparation of raw drugs

Heartwood of *Khadira* was procured from the forest of Moti Panchasara near Jamnagar in the month of December, 2014. After proper Authentication it was subjected to size reduction (*yavakuta* Form). Other ingredients were procured from the pharmacy. Baking yeast was purchased from local market (Brand Name- Blue Bird, Mfg- Sep. 2014, Exp. Jun 2015 and Batch No- S005). R.O. water was used in the preparation of *Kwath*

2.2 Preparation of Kwatha

In classics, the ratio to prepare the *Kwatha* of *Khadira* is not mentioned, so as a general principle, considering *Khadira Sara* (heartwood) as a very hard material, to prepare the *Kwatha*, sixteen times water was used and reduced up to 1/4th [5]. Before that, the *yavakuta churna* of *Khadira Sara* had been soaked overnight (12 hours) in RO water.

2.3 Preparation of Wort

The sweetening agent is also not found mentioned. So on trial and error basis, with reference from other related *Asava Arista* preparations, the proportion of sweetening agent was decided as per the general principle advocated by *Acharya Sarangdhara* [5].

2.4 Preparation of Kanakabindvarishta

Before preparation of the formulation for fermentation, the fermenting vessels (Porcelain Jars) were properly washed with washing soda/detergent, rinsed well with sufficient quantity of hot water. After proper cleansing, the vessels were completely dried to avoid any contamination. Vessels were subjected to sterilization by *Dhoopana* with *Guggulu* and *Aguru* [6].

Khadira Kwatha was taken in a large stainless steel vessel and allowed to self-cooling up to room temperature. Jaggery and honey was added to *Kwatha* in small quantities with continuous stirring to ensure its proper dissolution, before adding additional jaggery and honey. After complete dissolution of jaggery and honey, *Prakshepa* was mixed well in solutions to prepare wort. After preparing the wort, little quantity was taken in another vessel and yeast was dissolved in it. After starting the growth of yeast, the mixture was added back to wort from which it was initially taken. The wort was filled in to the properly cleaned, dry fermentation vessel ensuring 1/4th of the top space of the vessel vacant. Lid was closed properly, labelled and vessel was placed in clean, dry, moisture free and properly fumigated sterilized room to resist direct exposure of sunlight, air and variation in the atmospheric temperature.

After getting the positive completion signs of the fermentation, the supernatant fluid was decanted in another properly clean, dried porcelain jar after straining through a double folded cotton cloth. The marc remained in the bottom of the vessel was discarded.

2.5 Precautions:

Proper drying of the raw drug was needed before subjecting it to size reduction. Soaking should be done for overnight (approx. 12h) for uniform extraction. Mild heat should be provided maintaining the menstruum temperature in between 84 - 92 °C. Continuous stirring is needed. Dry superficial agglomeration of the powder added over the liquid may work as the base material for unwanted microbial growth, thus proper mixing with stirring was needed to facilitate proper absorption of the liquid inside the tissues of the drug. The jaggery, honey and *Prakshepa Dravya* should be mixed in the *Kwatha* at room temperature. Sealing of the vessel should be done properly and not allowed to disturb the vessel during the fermentation process.

3. Observation and Result

All the samples of *Kanakabindvarishta* were subjected to organoleptic and physico- chemical studies in order to develop analytical profile. The following parameters were carried out in this phase:

- Organoleptic characteristics: Colour, odour, touch and taste. (Table 4)
- Physico- chemical analysis: Loss on drying at 110° [7], pH value [8], water soluble extractive [9], methanol soluble extractive [10], determination of sugar contents [11], (Table 5, Table 6, Table 7)
- Qualitative test for various functional groups [12,13] : (Table 8)
- Microbial overload [14]: The tests were carried out at Shrey Pathology Laboratory, Jamnagar, Gujarat. (Table 9)

4. Discussion

Water is used as a solvent (menstruum) for extraction. Water is a solvent of proteins, colouring matters, gums, anthraquinone derivatives, most alkaloid salts, glycosides, sugars and tannins [15]. So before the preparation of *khadira kwatha*, *khadira yavakuta* was soaked in the water for approx. 12 hours.

Almost all the batches of *Khadira Kwatha* were prepared in average 18.6 h of duration. (Table 2) It was a sufficient time for enzymatic action between solvent (water) and solute (soluble of *Khadira Yavkuta*) through diffusion and osmosis. Some actions may occur during prolongation of heat treatment. Hydrolysis is one of the great reactions. Foam is also produced by elevation of temperature and agitation due to saponin content.

Fermentation started and ceased earlier because of addition of yeast. In the present study, adjuvant had a great part in the formulation; it absorbs from wort and gives marc in great quantity. The yield was found in between 58.92 to 68.60 %. The yield may be less due to more quantity of *Prakshepa Dravyas*. It means it occupies almost 1/3rd part of total wort. It increases total solid substance of the wort and after fermentation marc in total volume. 0.3% dry yeast was added in wort. This amount of yeast initiated fermentation activity earlier and hampers growth of unwanted microorganism, but it could not complete fermentation activity earlier. It took average 57 days for completion of fermentation activity. This higher time period required for completion might have very well contributed in increased biomass / therapeutically active principles. (Table 3)

5. Analytical Study

Organoleptic characteristic changes in its step of preparation of *Kanakabindvarishta*. After completion of fermentation, the fermented liquid became light. Utilization of honey (Almost 95%) may lead to lighter colour in end product. After completion of fermentation all batches of *Kanakabindvarishta* were same in colour. Break down of sugars and conversion of sugar may lead to the thinness in consistency of end product.

pH of *Kwatha* gradually fall in wort and *Kanakabindvarishta*. It shows acidic nature of all. Range of pH 3 – 5 is considered as most favourable condition for growth and fermentation activity of yeast. Further decrease in pH of *Kanakabindvarishta* attributes to the fermentation of ethyl alcohol.

Total solid content was less in yeast added wort may be due to activity started by yeast earlier. Addition of jaggery and honey cause increase in specific gravity of *Kwatha*. Decrease in total solid contents & specific gravity in finished product indicates utilization of sugar.

Raw drug *Khadira* collected from Saurashtra regions 7.1% of tannin respectively. Many references show the different percentage of tannin in the *Khadira* Heartwood ranging from 06% to 15%. Less % of tannin found in Saurashtra Region as it

depends upon the habituate and the maturity of the plant. Older the plant, more % of tannin content may be found because of more annual rings and tylosis formation [16]. Tannin percentage of final product was 4.09% that shows utilization of tannin in fermentation process.

In final product, Alcohol percentage was found in between 5.04 to 9.76. Reducing sugar was in between 22.81 to 25.15, non-reducing sugar is in between 3.44 to 4.21. Total sugar is in between 26.25 to 28.86. Utilization of total sugar shows resulting in more alcohol percentage. All physico-chemical parameters of all batches of *Kanakabindvarishta* have less variation. Presence of various functional group shows that in raw drug *Khadira* starch and steroid are not present, while in all batches of *Kanakabindvarishta*, starch is not present. It shows steroid comes from another source. It may be from

other raw drugs or intermediate procedure.

Limit Test for Microbial overload in all the batches of *Kanakabindvarishta* was under prescribed limit and Methanol was not present in all the batches. It was found to be safe from microbial overload and absence of methanol side.

6. Conclusion

The S.M.P has been developed to prepare *Kanakabindvarishta* by using yeast as a fermenting agent. Sixteen times water is found sufficient to prepare *Kwatha* by considering *Khadira Sara* as a very hard material. General ratio of sweetening substance has been followed as described by *Acharya Sharangdhara*. The physico-chemical parameters may be taken as Quality control parameters for future studies.

Table 1: Ingredients and their proportions in *Kanakabindvarishta* in each batch

Dravya	Drugs	Proportion
<i>Kwathya Dravya</i>	<i>Khadira</i>	3.870kg
<i>Prakshepa Dravya</i>	<i>Triphala, Trikatu, Vidanga, Haridra, Darvi, musta, Vasa, Indrayava, Guduchi, Tvaka</i>	360g each
<i>Madhura Dravya</i>	<i>Guda</i>	3 kg
	<i>Madhu</i>	1.5 kg
<i>Sandhana Dravya</i>	Dried Yeast	35 g

Table 2: Comparative Pharmaceutical details of the preparation of *Kwatha*

Observations	Avg of observation (n=5)
Amt. Of <i>Khadira Yavakuta</i> (Kg)	3.870
Quantity of water(L)	62
Room Temperature (°C)	20-28
Flame Temperature (°C)	290-360
Total duration (h)	18.6
Total yield (L)	15.2

Table 3: Comparative Pharmaceutical details of the preparation of *Kanakabindvarishta*

Parameters	Avg of observation (n=5)
Yield of <i>Kwatha</i> (l)	15.000
Amt of Jaggery added (kg)	03
Amt of Honey added (kg)	1.5
Total amt. of <i>Prakshepa dravyas</i> added (kg)	3.6
Volume made of solution of Jaggery Honey & <i>Kwatha</i> (l)	21.77
Fermentation started in (days from placement)	5.2
Fermentation completed in (days from placement)	57
Yield of <i>Kanakabindvarishta</i> (l)	13.34
Percentage of yield (%)	61.26

Table 4: Comparative organoleptic characters of *khadira* heartwood, Soaking, *Kwatha*, and *Kanakabindvarishta*

Parameters	<i>Khadira</i>	Soaking(12h)	<i>Kwatha</i>	Wort	<i>Kanakabindvarishta</i>
<i>Rupa</i> (Colour)	Light Red	Light orange	Reddish brown	Brown	Dark reddish brown
<i>Rasa</i> (Taste)	Taste-less	Taste-less	Kashaya	Madhura Kashaya Tikta	Kashaya, madhura, Tikta
<i>Gandha</i> (Odour)	Characteristic	Characteristic	characteristic	Fruity sweet	Fruity alcoholic
<i>Sparsa</i> (liquid consistency)	Powder	Thin	Thin	Thick	Thin

Table 5: Comparative Physico-chemical Analysis of *Khadira* (Acacia catechu Willd. Heartwood)

Tests	<i>Khadira</i> Heartwood
Loss on drying at 110 C	9.00%
Total ash	9.0%
Acid insoluble ash	0.7%
Water soluble extractive	14.97%
Methanol soluble extractive	13.06%
pH of Water extract	5.33%
Quantitative estimation of tannin	7.1%

Table 6: Comparative determination of pH, Total solid content and specific gravity of *Kwatha*, wort and *Kanakabindvarishta* (n=5)

Groups	pH	Total solid content	Specific gravity
<i>Kwatha</i>	5.15	4.31	1.02
Wort	4.59	63.00	1.16
<i>Kanakabindvarishta</i>	3.28	16.71	1.1

Table 7: Determination of some analytical parameters of *Kanakabindvarishtha* (n=5)

Parameters	(Mean n= 5)
Water soluble extractive	16.16
Alcohol soluble extractive	21.52
Alcohol content	6.52
Reducing Sugar	23.94
Non Reducing Sugar	3.79
Total Sugar	27.73
Quantitative estimation of tannin	4.09%

Table 8: Determination of presence of various functional groups of all the batches of *Kanakabindvarishtha* and raw drug *Khadira* (n=5)

Parameter	<i>Khadira</i>	<i>Kanakabindvarishtha</i>
Alkaloid	+	+
Flavonoids	+	+
Starch	-	-
Tannin	+	+
Saponin	+	+
Steroid	-	+
Carbohydrate	+	+
Protein	+	+

Table 9: Determination of Qualitative test for methanol, ethanol and Microbial growth in all batches of *Kanakabindvarishtha* (n=5)

Sample	<i>Kanakabindvarishtha</i>
Methanol	-Ve
Ethanol	+Ve
Bacterial growth	-Ve
Fungal growth	-Ve

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