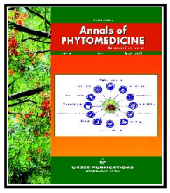
Annals of Phytomedicine 10(1): 160-170, 2021

DOI: http://dx.doi.org/10.21276/ap.2021.10.1.16

Annals of Phytomedicine: An International Journal

http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original article: Open access

Standardization of beejakarishta preparation with respect to seasonal changes and the determination of its antibacterial activity against selected clinical isolates

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Article Info

Article history
Received 12 April 2021
Revised 29 May 2021
Accepted 30 May 2021
Published online 30 June 2021

Keywords
Beejakarishta
Sandhana kala
Fermentation tests
Antibacterial activity
Multi drug resistant (MDR)
Bacteria

Abstract

Beejakarishta is an 'arishta' (self-generated alcoholic) preparation in Ayurveda with a wide range of pharmacological applications including in digestive disorders, anaemia, piles, inflammation, urinary tract infections (UTIs), diabetes, jaundice, *etc*. Even though, there are many studies which show the effect of different containers and method of preparation in the fermentation process of different arishta-asavas (fermented ayurvedic potion); no studies have been conducted to analyse the effect of different seasons and different time duration on the physicochemical characteristics and pharmacological potentials of beejakarishta. So, present study investigated the effect of season and duration in fermentation process of beejakarishta and variation in their analytical properties. In this study, six samples are made in April (summer season–greeshmaritu), October (autumn season - sarataritu) and in January (winter season -sisiraritu). In each season, three samples each were kept for 10 days fermentation and for 20 days fermentation. Fermentation tests and physicochemical analysis were carried out for all the samples and compared. HPTLC analysis was performed to evaluate the compounds present in the test samples. Beejakarishta with 20 days fermentation in summer season (BAG_{20}), which showed typical physicochemical properties is examined for antibacterial analysis. 100 µl of BAG_{20} showed significant antibacterial activity against *Escherichia coli* (18 mm), *Staphylococcus aureus* (19 mm) and *Klebsiella pneumonia* (26 mm). From the study, it was confirmed, seasons have a greater impact in determining the properties of the beejakarishta. The BAG_{20} can be developed as an effective novel antibacterial drugs for treatment of multidrug resistant (MDR) infection.

1. Introduction

Bhaishajyakalpana is the pharmaceutical branch dealing with various types of medicinal preparation as reported in ayurveda. Sandhana-kalpana (biomedical fermentation) is a unique dosage form of ayurvedic pharmaceutics. It comes under secondary preparations which are based on primary formulations like swarasa (juices) kwatha (decoctions) *etc*. Sandhana is a process, in which liquid preparations either along with medicines or food materials are kept in a vessel for long duration to facilitate the fermentation process (Yadavji Trikamji, 1983). Based on the end product formed, sandhanakalpana is divided in to madyakalpana (where the end-products are alcoholic preparations like arishta, asava, *etc*.) and shukatakalpana (where the end-products are the preparations of acidic nature like dhanyamla). Among these, arishta is said to be superior as its unique combination of drugs and processing (Krishnamurthy, 1973). These formulations have longer shelf life, quick absorption and action and excellent therapeutic efficacy (Parasurama Sastri, 2008).

Beejakarishta has been recommended in wide range of clinical conditions like digestive disorders, anaemia, piles, inflammation, urinary tract infections (UTIs), diabetes, jaundice, *etc*. (Sre Ganga Sahya Pandeya, 2003). Even though, various Acharyas explained different seasons and time periods, most of the references of beejakarishta explain fermentation period as ten days in summer season and 20 days in winter season (Srikantha Murthy 1984; Murthy Srikantha, 2009 and Sharma Ram Anant, 2012).

Fermentation process depends upon many factors like nature and quantity of the ingredients, methods of preparation, the containers for fermentation, place of fermentation, seasons, duration of fermentation, *etc*. (Harisankar Pandey, 1993). Among these, duration of fermentation and seasons have a major role in progress of fermentation. There are studies which show the effect of different containers and method of preparation over the fermentation process of different arishtas and asavas, but no study has been conducted with respect to duration and seasonal effect. The duration for fermentation vary from formulation-to-formulation and in general, 30 days is explained in classic fermentations with some exceptions. In beejakarishta, it is specified that, ten days in summer and 20 days in winter are the optimal durations. This indicates season has a role to determine the duration of fermentation and in classical references, the months of January and February are considered as winter (sisira), April and May as summer (greeshma) and October and November as autumn (sarat) (Sir Monier-Williams, 1899, Visvanatha Dvivedi Shastri, 2002).

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The wide spread of multidrug resistant (MDR) bacteria is one of the global challenges of the 21st century. MDR microbes are generally resistant to three or more antibiotics. Despite of the development of new antibacterial agents, the past three decades have been witnessing for an increase in resistance against drugs by bacteria. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Thus, there is a need for development of new antibacterial agents from different sources (Elbossaty, 2017; Aslam *et al*., 2018).

The present study examines, the changes in beejakarishta prepared in different seasons with different duration of fermentation (10 and 20 days) and determines the antibacterial activity of the most efficient sample against four of the clinically isolated pathogens, *viz*. *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia* and *Acinetobacter baumannii.* Fermentation tests and physicochemical analysis were carried out for all the samples and compared. HPTLC analysis was performed to evaluate the compounds present in the test samples.

2. Materials and Methods

2.1 Collection of raw materials for the study

The major ingredients (Table 1) were collected from raw drug unit of MVR ayurvedic pharmacy and authenticated by Department of Pharmacology, MVR Ayurveda Medical College, Kannur. Honey was collected from Eramam, Kannur and subjected to different analytical parameters. Raw brown sugar was collected from farmers at Bangalore, Erode and Coimbatore and the quality was confirmed before the usage.

Table 1: Ingredients of decoction (kashaya)

Sl. No.	Ingredient	Scientific name	Part used	Quantity taken for the preparation of 1 sample
1	Asana	*Pterocarpus marsupium*	Heart wood	256 g
2	Hareethaki	*Terminalia chebula*	fruit	106 g
3	Vibheethaki	*Terminalia bellirica*	Fruit	106 g
4	Amalaki	*Phyllanthusemblica*	Fruit	106 g
5	Draksha	*Vitisvinifera*	Fruit	80 g
6	Laksha	*Lacciferlacca*	Resin	112 g
7	Water			4 lit

2.2 Preparation of beejakarishta

2.2.1 Poorva karma (pre-procedure)

Six glazed porcelain jars of three-liter capacity were taken. Each were cleaned thoroughly and dried under hot sun for two days. The inside of jar is then fumigated using sandal, vetiver, camphor, long pepper, spikenard and pepper. Then, the inner surface of the vessels was smeared with ghee and kept aside. For condiments (Table 2), the drugs were taken in required quantity and separately pounded in mortar to a coarse powder.

2.2.2 Pradhana karma (main procedure)

The required drugs (as given in the Table2) were properly cleaned and dried. They were pounded to coarse powder separately. The drugs were put in a steel vessel. 1 liter of water was added and the level of water was noted. Then, 3 more liters of water added, placed on mild fire, boiled, frequently stirred till the water reduced to 1/4th. Then, it was strained and cooled to room temperature. Sugar was added little-by-little and dissolved completely. Then, it was strained through a cloth followed by the addition of honey and thoroughly mixed. The mixture is poured carefully into the prepared container. It is filled up to three fourth capacity of the container. Condiments are added and stirred well**.** The mouth of container is closed with a piece of cloth, and then the lid is placed above that. Sealing is done by winding a three layered cloth around joint which is smeared with clay (Figures 1 to 5).

2.2.3 Paschat karma (following procedure)

The filled containers were kept in a cardboard box filled with barley up to the neck portion. Following the same procedure, six samples are prepared in summer, autumn and winter seasons. Fermentation tests were observed by changes in state of prak sepakadravya (condiments) effervescence, hissing sound, alcoholic odor and taste, burning candle test, **l**ime water test. In each season, 3 samples were kept for ten days fermentation, and on 11th day they were opened and the product was filtered through a starch free muslin cloth. Other 3 samples were kept for 20 days fermentation and on 21st day they were opened and filtered. The products were then subjected to physicochemical analysis (Figures 6 to 8).

Table 2: Ingredients of condiments (prakshepa)

Sl.No.	Ingredients	Scientific name	Part used	Quantity taken for the preparation of 1 sample
1	Nagara	*Zingiber officinale*	Rhizome	4 gm
2	Maricha	*Piper nigrum*	Fruit	4 gm
3	Pippali	*Piper longum*	Fruit	4 gm
4	Vyaghranagha	*Capparis zeylanica*	Plant	4 gm
5	Usheera	*Vetiveria zizanioides*	Root	4 gm
6	Kramuka	*Areca catechu*	Fruit	4 gm
7	Elavaluka	*Prunus cerasus*	Fruit	4 gm
8	Madhooka	*Madhuca longifolia*	Flower	4 gm
9	Kushta	*Saussure acostus*	Root	4 gm

Figure 1: Kashaya Preparation.

Figure 2: Adding sharkara.

Figure 3: Adding kshuodra.

Figure 4: Dhupana of vessel.

Figure 5: Ghritalepana.

Figure 6: Transferring to the container.

Figure 7: Adding prakshepa.

Figure 8: Placing for sandhana.

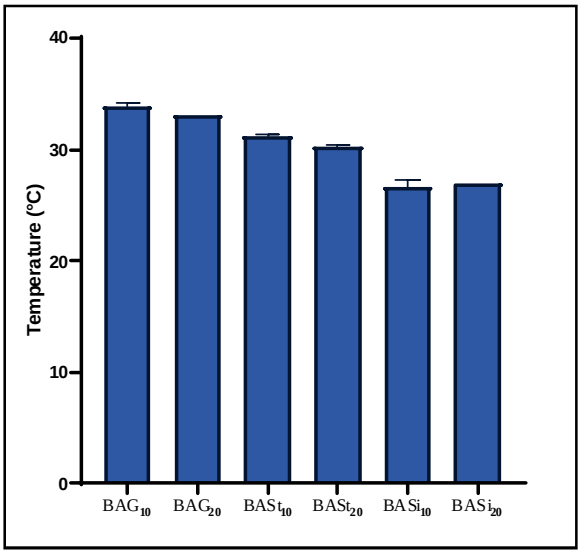

Figure 9: Analysis of temperature.

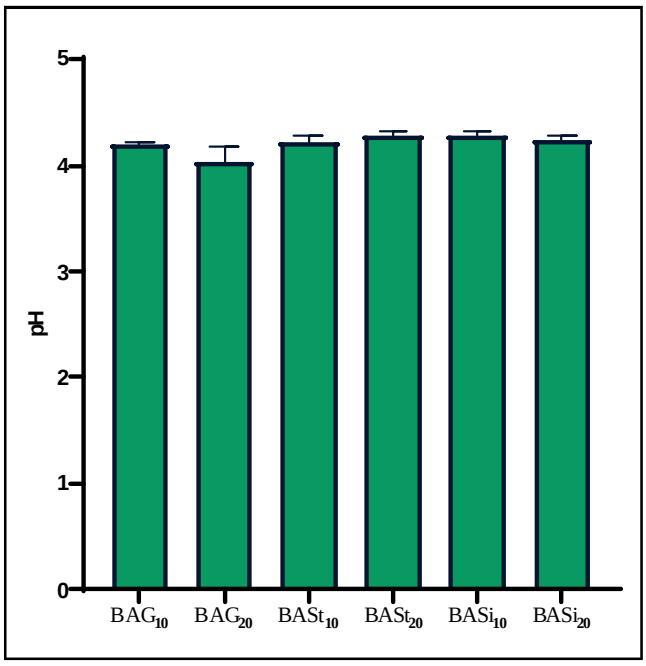

Figure 10: Analysis of pH.

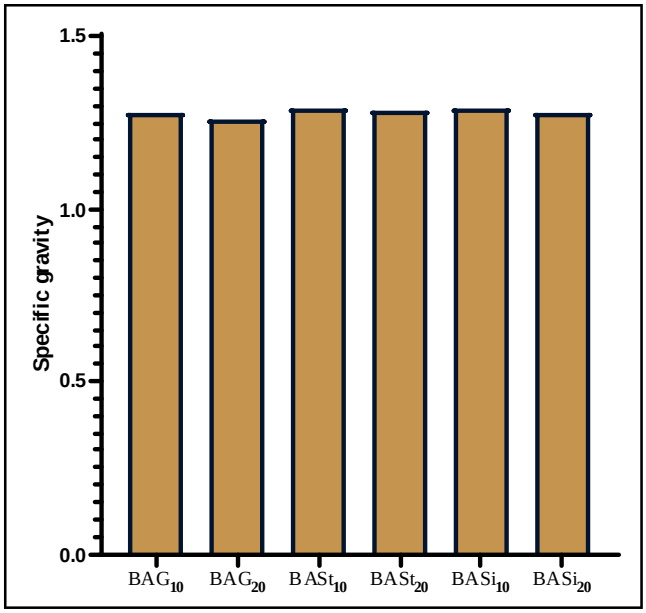

Figure 11: Analysis of specific gravity.

2.3 Analysis of beejakarishta

The prepared samples of beejakarishta were analyzed for its physical and chemical properties. The physical tests included burning candle test, lime water test, temperature, pH, specific gravity, total solids, brix, alcohol content, *etc*., and the chemical tests included total sugar, reducing sugar, non-reducing sugar, total acidity, *etc*.

2.4 High performance thin layer chromatography (HPTLC) analysis

The analysis was performed at win CATS Planar Chromatography Manager, CAReKeralam Ltd., Thrissur, India. 10 ml of test sample

was partitioned in a separating funnel with 20 ml of methanol extract. The hexane soluble portion was evaporated at room temperature to a volume of 1 ml. 8 µl and 12 µl of above sample was applied on a pre-coated silica gel on aluminum plates to a band width of 8 mm using Linomat 5 TLC applicator. The plate was developed in toluene ethyl acetate (8:2) and the developed plates were visualized under UV 254 nm, 366 nm. The data like Rf values, densitometric scan, colors of the spots, *etc*., were observed and recorded.

Table 3: Description of samples

Sl.No.	Time duration and season	Sample1	Sample2	Sample3
1	10 days in summer	$BAG_{10}1$	$BAG_{10}2$	$BAG_{10}3$
2	20 days in summer	$BAG_{20}1$	$BAG_{20}2$	$BAG_{20}3$
3	10 days in autumn	$BASt_{10}1$	$BASt_{10}2$	$BASt_{10}3$
4	20 days in autumn	$BASt_{20}1$	$BASt_{20}2$	$BASt_{20}3$
5	10 days in winter	$BASi_{10}1$	$BASi_{10}2$	$BASi_{10}3$
6	20 days in winter	$BASi_{20}1$	$BASi_{20}2$	$BASi_{20}3$

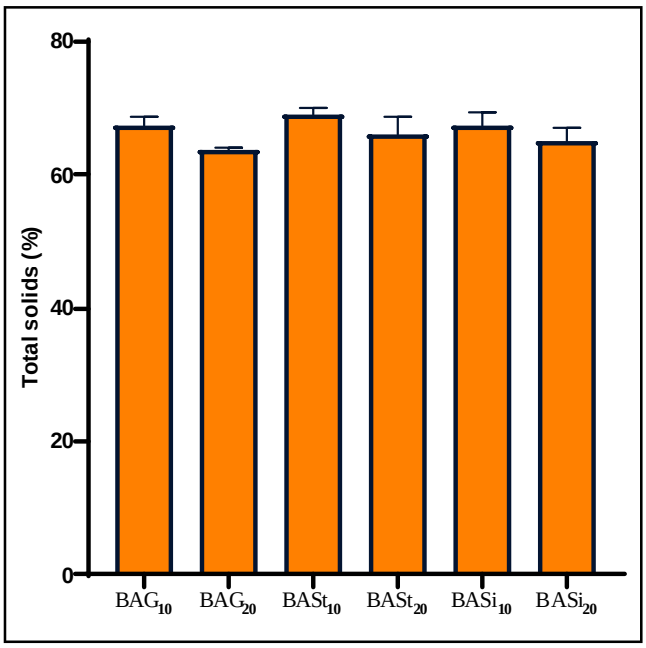

Figure 12: Analysis of total solids.

2.5 Antibacterial analysis

The prepared beejakarishta was subjected to antibacterial activity against hospital acquired pathogens. Antibacterial activity was determined by using well diffusion method (Rajesh *et al.*, 2014) against *Escherichia coli, Staphylococcus aureus, Klebsiella pnerumoniae* and *Acenitobacter bauumanni.* Briefly, nutrient agar plates were prepared and overnight cultures of test organisms were swabbed over the plates. 6 mm well borer was used to bore wells. 3 concentrations of the samples were added to each well and other well was loaded with standard drug (ciprofloxacin). The plates were incubated at 37°C for 48 h. After incubations, the plates were observed for inhibitory zones. The zones were measured in millimetres.

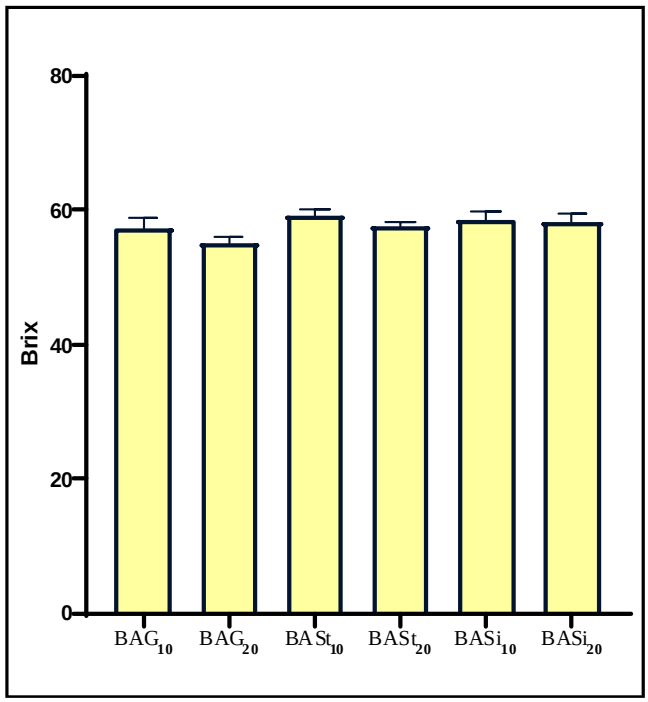

Figure 13: Analysis of brix.

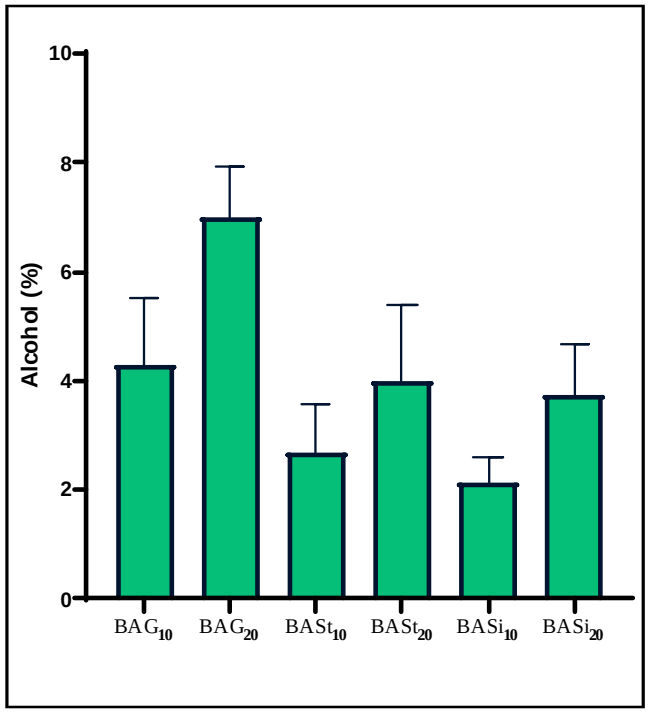

Figure 14: Analysis of alcohol content.

3. Results

Fermentation analysis, floating of praksepakadravya (Table 3) - condiments - effervescence, hissing sound, burning candle test, lime water test. The fermentation analysis included the steps described below.

Table 4: Comparison of Rf values and area percentages of the samples

	G-10	G-20	ST-10	ST-20	SI-10	SI-20
-0. 07	+ (91.81%)			+ (88.76%)	+ (88.12%)	+ (87.47%)
-0. 06		+ (86.32%)	+ (85.16%)			
0. 06					+ (8.49%)	+ (9.76%)
0. 07			+ (10.37%)	+ (10.51%)		
0. 09		+ (8. 72%)				
0. 10	+ (6. 20%)					
0. 59					+ (3.39%)	
0. 62						+ (2.77%)
0. 64		+ (0. 93%)				
0. 71				+ (1.73%)		
0. 95			+ (4.46%)			
0. 97		+ (4.03%)				
0. 99	+ (2. 00%)					

The plus sign indicates the presence of a peak in the range mentioned. The percentage values with respect to their concentrations have been provided in brackets. Left column lists the Rf values.

Table 5: Antibacterial activity of beejakarishta (20 days'/summer season)

Sl.No.	Test organisms	Inhibitory zones (mm) against quantities			
		25 µl	50 µl	100 µl	Std. drug
1	*Escherichia coli*	-	-	18	24
2	*Staphylococcus aureus*	-	15	19	21
3	*Klebsiella pneumonia*	-	10	26	29
4	*Acenitobacter bauumanni*	-	-	-	23

3.1 Floating of praksepakadravya (condiments)

By fermentation aqueous media change to alcoholic media which is less dense than water. So, the sunken condiments indicate the production of alcohol. All the samples in summer season, autumn and winter season showed floating of condiments from 0^{th} day to 11^{th} day. Summer season samples gradually began to sink and on 21^{st} day in $BAG_{20}1$ and in $BAG_{20}2$ condiments were partially sunken, and in $BAG_{20}3$, they were mostly sunken. On 21^{st} day, in autumn season and winter season, the condiments partially floated and partially sunken in all samples which indicated the production of alcohol.

3.2 Effervescence

The formation of CO_2 during process caused bubbling in the liquid and was observed in 5^{th} and 11^{th} day, analysis of summer season samples. On 11^{th} day large bubbles were seen, showing progress of fermentation. On reaching 20^{th} day, no effervescence in $BAG_{20}1$ and3 sample and decreased considerably in $BAG_{20}2$. In autumn and in winter samples, the effervescence was present on 5^{th},11^{th} and 21^{st} days but decreased in $BASt_{20}$ samples.

3.3 Hissing sound

The formation of CO_2 makes the hissing sound in summer season samples, it was absent in 0^{th} day but pre sent in 5^{th} day and 11^{th} day, and again absent in 21^{th} day, indicating gradual progression and completion of fermentation. Inautumn season samples, it was present in 11^{th} day and 21^{st} day but decreased in 21^{st} day. In winter season, hissing sound was absent in 0^{th},5^{th} day but present in 11^{th} day and 21^{st} day, indicating continuation of fermentation.

3.4 Burning candle test

In summer season and autumn season, burning candle was put off in 5^{th} day and 11^{th} day, but continues to burn on 21^{st} day. In $BASt_{20}3$, it was put off in 21^{st} day. In winter season, candle continued to burn 5^{th}, and put off on 11^{th} and 21^{st} day. During the process of fermentation, burning candle got extinguished if taken near to the fermentation liquid surface. This was because of the production of CO_2 by fermentation. After completion of fermentation, the candle continued to burn since there was no CO_2 produced. During the process of fermentation, burning candle get extinguished if taken near to the fermentation liquid surface. This is because of the production of CO_2 by fermentation. After completion of fermentation, since no CO_2 is produced the candle continues to burn.

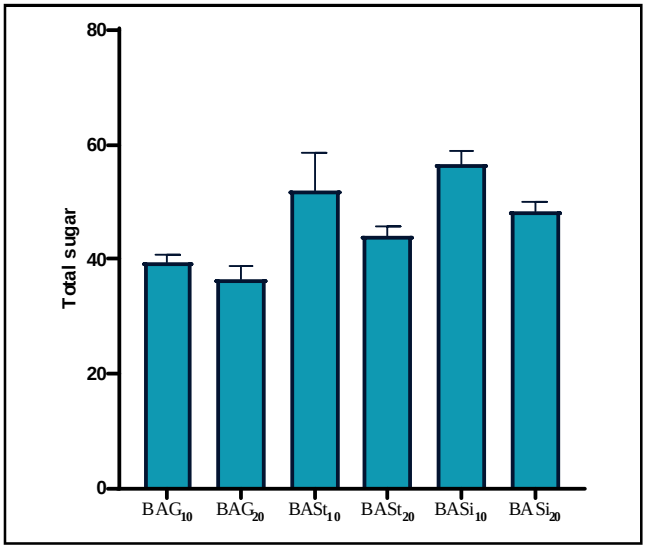

Figure 15: Analysis of total sugar.

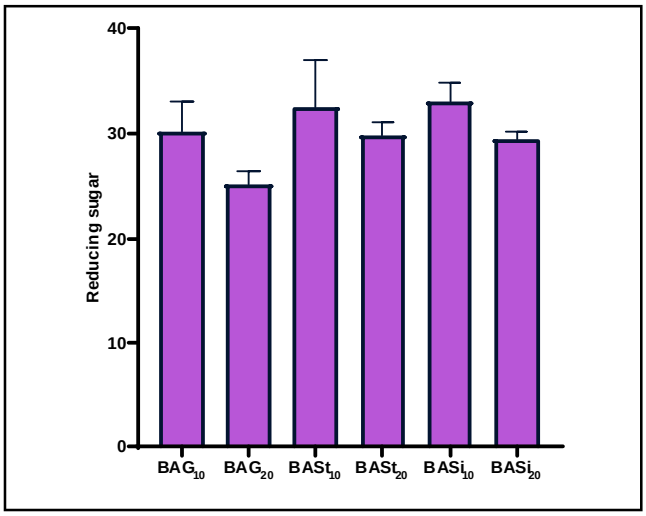

Figure 16: Analysis of reducing sugar.

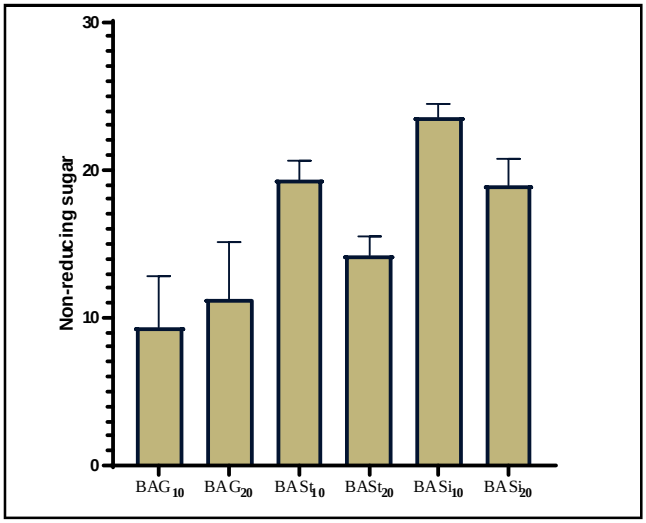

Figure 17: Analysis of non-reducing sugar.

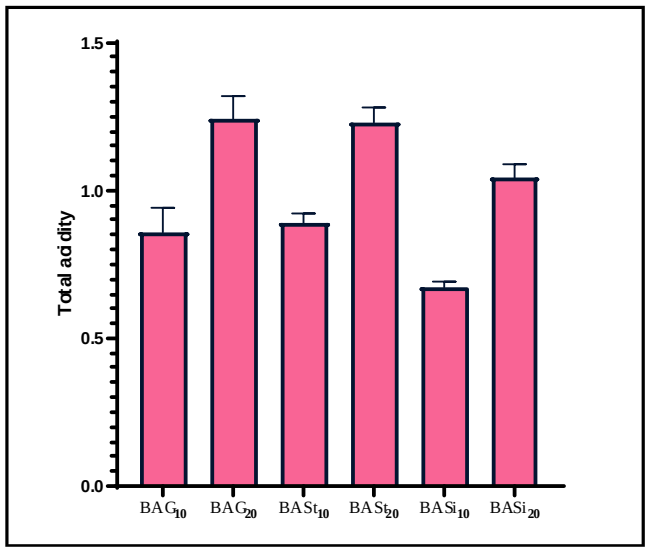

Figure 18: Analysis of total acidity.

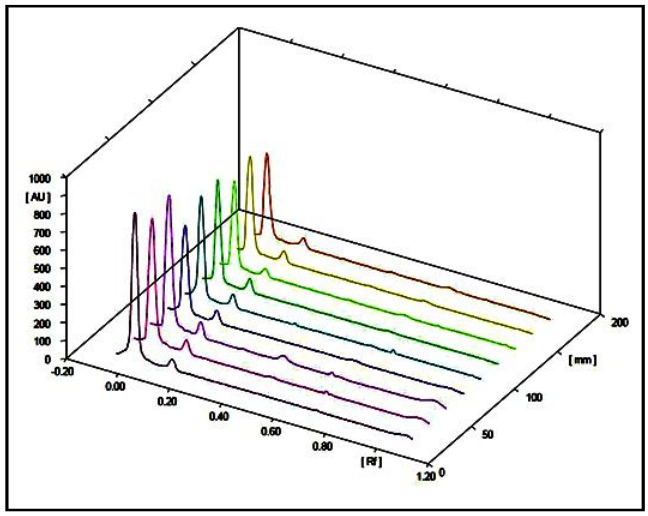

Figure 19: Graphical representation of overall track analysis.

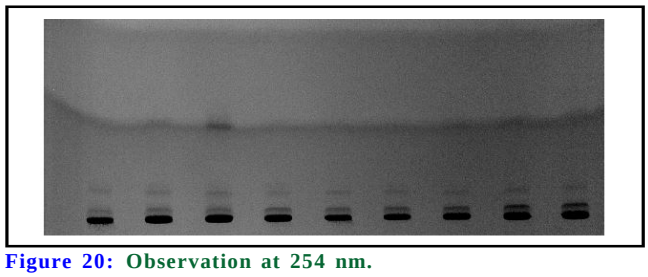

Figure 20: Observation at 254 nm.

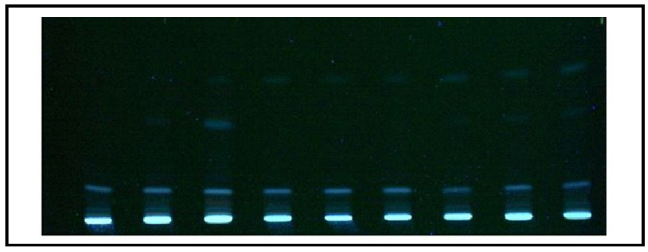

Figure 21: Observation at 366 nm.

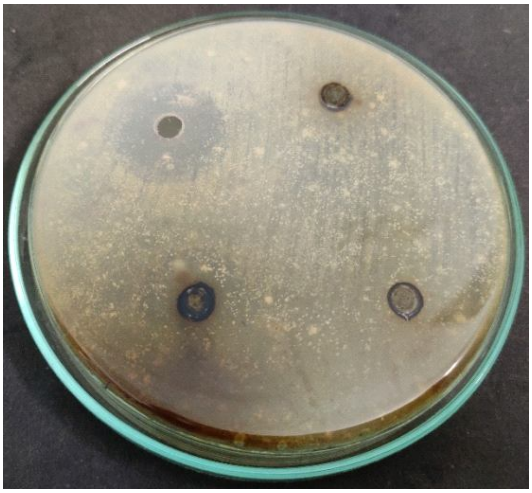

Figure 22: ABA against *E. coli*.

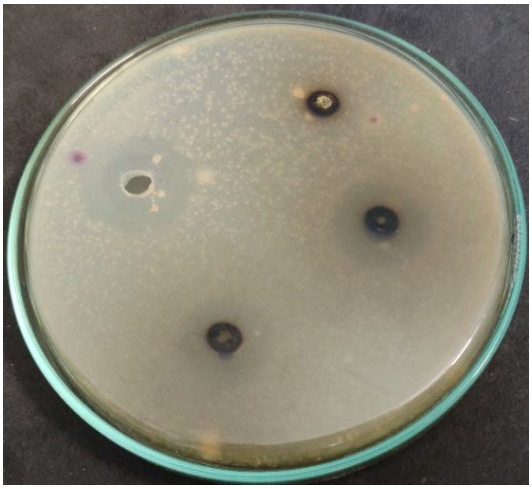

Figure 23: ABA against *S. aureus*.

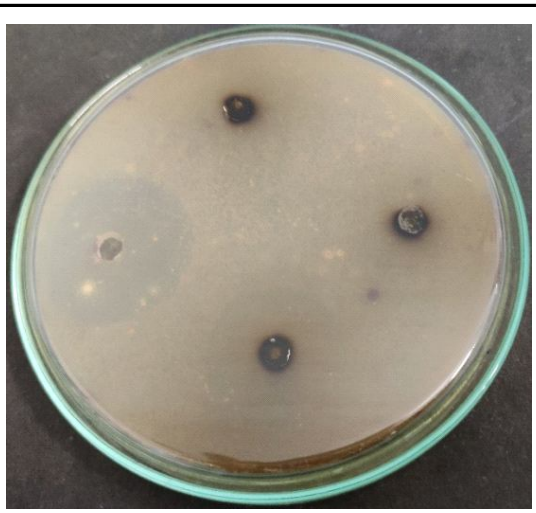

Figure 24: ABA against *K. pneumoniae*.

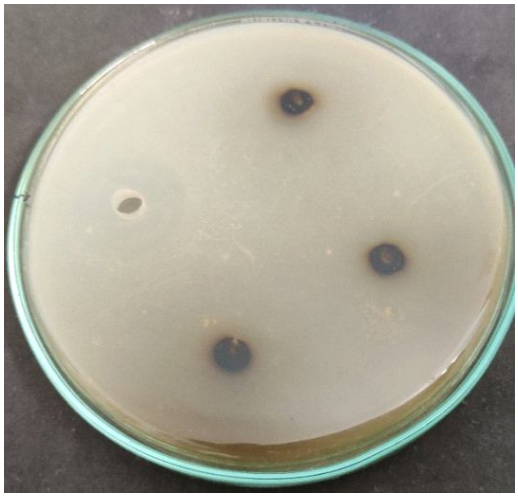

Figure 25: ABA against *A. baumanni*.

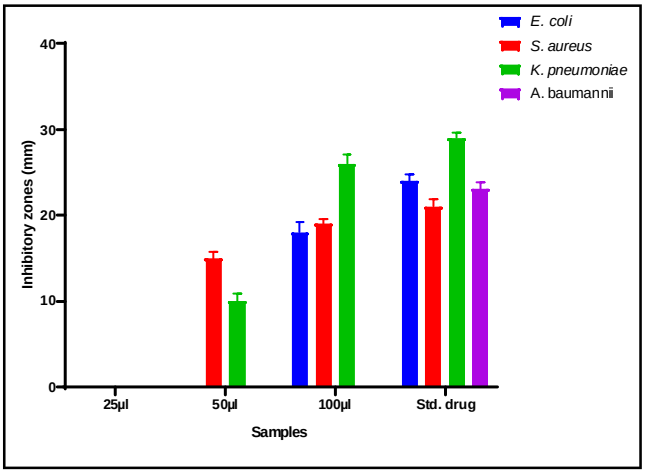

Figure 26: Antibacterial activity of beejakarishta of 20 days fermentation sample in summer season.

3.5 Limewater test

This test showed positive result for 5th and 11th day samples and negative results for 21st day sample in summer season and autumn fermentations. In winter season, it was negative in 5th and positive in 11th and 21stday. A positive test resulted when lime-water turned milky. It is due to the reaction of calcium hydroxide with carbon dioxide to form calcium carbonate which is insoluble in water and, thus forms a milky turbid appearance

3.6 Organoleptic characters

3.6.1 Appearance

The samples were found to be thick liquid on 0th day and frothy liquid on 5th day and on 11th day. On 21st day, the samples were less frothy except samples on winter season.

3.6.2 Odor

No alcoholic smell was identified on 0th day. This indicates the initiation of fermentation. Alcoholic smell was felt on 11th day and the smell became intense on 21st day.

3.6.3 Taste

On 0^{th} day, the taste of the samples was found to be sweet, astringent and bitter. The fermentation has changed the taste to alcoholic on 5^{th} and 11^{th} day. The alcoholic taste was higher on 21^{st} day.

3.6.4 Color

The color of the samples was found to be dark reddish brown on 0^{th} day. There was no color change observed during fermentation on 5^{th}, 11^{th} and 21^{st} day.

3.6.5 Touch

The samples were found to be sticky and thick on 0^{th} and 5^{th} day, on 11^{th} day, the samples in summer season were observed to be thinner and sticky. The samples on 21^{st}day were found to be thinner and less sticky.

3.7 Analytical study

3.7.1 Temperature

The room temperatures were found to be 33°C for summer season, 30°C for autumn season and 26°C for winter season. The temperature had slight variation after fermentation for 10 and 20 days. This shows that external climate affects the fermentation process by altering the temperature (Figure 9). Optimum temperature in the range of 20-35°C is suitable for initiation of fermentation. The growth rate of yeast cells is strongly influenced by fermentation temperature. The rate of fermentation is slowed down in cooler temperatures as they prolong the lag phase. At low temperatures, yeasts tend to be less sensitive to the toxic effects of high alcohol concentration. In the case of different yeasts, the higher (warmer) temperatures, a rapid decrease in cell viability is noticed. When the temperature is becoming very high, the functions of enzymes and the integrity of membranes are affected, which could result a halt in the fermentation process. But, in fact, the optimum temperatures for different yeast fermentations will vary as various strains respond in different ways to temperature.

3.7.2 pH

The pH of the samples varied between 4.02 ± 0.16 to 4.27 ± 0.05. The pH was low for BAG_{20} and highest was observed for $BASt_{20}$ and $BASi_{10}$. Figure 10 shows the pH of the samples used in the study. pH affects the shape of proteins in yeast cells which are responsible for the metabolic processes whereby fermentation. Each strain of yeast has an optimal pH range but most are in the range of 4 to 6. The initial pH is observed within this range, so it favored the imitation of fermentation. The reduction is more in summer season and less in autumn season. The pH may decrease during fermentation due to the production of carbonic acid (CO_2 dissolved in water) and other organic acids like gallicacid, citricacid, ascorbic acid, *etc*. Also, alcohol produced by fermentation may be another reason.

3.7.3 Specific gravity

The specific gravity of the samples varied between 1.259 ± 0.00 to 1.29 ± 0.00. The specific gravity was low for BAG_{20} and highest was observed on $BASt_{10}$. Figure 11 shows the specific gravity of the samples used in the study. This lowering may be due the breakdown of sugars into alcohol. By 20 days, more sugars may be converted to alcohol than 10 days and also the rate of breakdown may be less in autumn season, and more in summer season.

3.7.4 Total solids

Total solids content includes solid matter which is suspended, dissolved, or settled in a liquid, and are left after evaporation and drying of a sample. The total solids suspended in the samples varied between 63.66 ± 0.57 to 69 ± 1.00. The specific gravity was low for BAG_{20} and highest was observed for $BASt_{10}$. Figure 12 shows the total solids of the samples used in the study. Total solids decreased by increasing duration of fermentation. summer and autumn sample shows more reduction on 20^{th} day than winter samples.

3.7.5 Brix

The degree of brix in the samples varied between 55 ± 1.00 to 59 ± 1.00. The brix was low on BAG_{20} and highest was observed on $BASt_{10}$. Figure13 shows the brix of the samples used in the study.

3.7.6 Alcohol content

The alcohol content in the samples varied between 2.13 ± 0.46 to 6.98 ± 0.94. The alcohol production was low on $BASi_{10}$ and highest was observed on BAG_{20}. Summer season samples had more alcohol content. Figure 14 shows the alcohol content of the samples used in the study. On comparison, the formation of alcohol is more in summer samples and less in winter samples.

3.7.7 Total sugar

The total sugars present in the samples varied between 36.53 ± 2.44 to 56.66 ± 2.3. The total sugar was low on BAG_{20} and highest was observed on $BASi_{10}$. Total sugar is reduced from 11^{th} day to 21^{st} day. The reduction is more in summer season samples. Figure 15 shows the total sugars present in the samples used in the study. Total sugars comprise all mono-and disaccharides which are reducing and non-reducing sugars. It was seen that total sugar is reduced in 20 days sample than 10 days sample in all the 3 seasons.

3.7.8 Reducing sugar

The total sugars present in the samples varied between 25.16 ± 1.2 to 33.12 ± 1.75. The reducing sugar was low on BAG_{20} and highest was observed on $BASi_{10}$. Reducing sugar is reduced from 11^{th} day to 21^{st} day. The reduction is more in summer seasonsamples. Figure 16 shows the reducing sugars present in the samples used in the study.

3.7.9 Non-reducing sugar

The non-reducing sugars present in the samples varied between 9.33 ± 3.41 to 23.54 ± 0.95. The non-reducing sugar was low on BAG_{10} and the highest was observed on $BASi_{10}$. Figure 17 shows the non-reducing sugars present in the samples used in the study.

3.7.10 Total acidity

The total acidity of the samples varied between 0.67 ± 0.02 to 1.24 ± 0.08. Figure 18 shows the total acidity of the samples used in the study. The overall track analysis is graphically represented in Figure 19.

3.7.11 HPTLC analysis

The HPTLC profiling, which was performed as described elsewhere in this report, using methanol sample solvent system, generated the results as shown Table 4, Figures 20, 21 indicate the Rf values, peaks and area percentage of samples.

3.7.12 Antibacterial analysis

Beejakarishta of 20 days fermentation in summer season (BAG_{20}) is used for antibacterial analysis. Three concentration (25 µl, 50 µl, 100 µl) of samples were used. Table 5 shows the inhibitory zones of the BAG_{20} against hospital acquired pathogens. 100 µl of BAG_{20} showed significant antibacterial activity against *Escherichia coli* (18 mm), *Staphylococcus aureus* (19 mm) and *Klebsiella pneumoniae* (26 mm). No inhibitory zones were obtained for *Acinetobacter baumannii* (Figures 22 to 26).

4. Discussion

The effectivity and efficiency of the antibiotics in use currently are being challenged greatly by the emergence of MDR bacterial strains. Moreover, the indiscriminate worldwide overuse and misuse of antibiotics is the major reason for the high rates of microbial resistance. A study estimates that about 33000 people die each year as a direct consequence of an infection due to bacteria resistant to antibiotics and that the burden of these infections is almost equal to the adverse effect of tuberculosis, influenza and HIV/AIDS combined. World Health Organization (WHO) had declared antimicrobial resistance (AMR) as an emerging crisis in the world and there is need for the development of new antimicrobial agents/therapies to overcome AMR (Aslam *et al.*, 2018). The present study focuses on development of beejakarishta at different conditions and evaluating its antibacterial activity against hospital acquired pathogens.

Beejakarishta mentioned in charakasamhitha is taken for the present study. Various references of beejakarishta was compiled and an attempt is made to understand the differences in the ingredients and preparation (Acharya Vaidya Jadavaji Trikamji, 2002 - 1). And all the ingredients including asana, which is the which is the major ingredient of beejakarishta (Acharya Vaidya Jadavaji Trikamji, 2002 - 2) were taken as described in charakasamhitha.

The name of yoga got from its synonym Beejaka. Acharya charaka included asana in 20 sarasava yoni Hareethaki and Amalaki included in 26 phalasava yoni.Vaghbhtachraya enumerated Draksha in 5 madya yoni and considered as best among them. Lac is the only animal origin resin used in Ayurveda. These kwathadravyas are pharmacologically and therapeutically much important in the Beejakarishta yoga as they are the main drugs. Most of them shows action in rasa raktha and medovahasrotas, kaphapithahara or sarvadoshaharakarma, rasayanaguna. Out of six, 4 are included in asava yoni. It may refer to the important of their asavarishta preparation (Acharya Vaidya Jadavaji Trikamji, 2002 - 2).

Therefore, the analysis of six samples of beejakarishta which were prepared in triplicates showed that, change in season affects the fermentation and physiochemical parameters varies. This confirms that temperature has a great impact in affecting the fermentation and altering its nature (Kushwaha Singh Chandra Harish,2011). Furthermore, the samples were subjected to HPTLC analysis to differentiate the bioactive compounds present in it. Several compound peaks were observed and specific peaks were observed in certain samples (G-20, ST-10 and SI-20). This indicates the, seasons play a vital role in affecting fermentation and deviation in bioactive compounds was pronounced (Murthy Srikantha, 2009). Moreover, the chemical compounds corresponding to the peaks need to be identified. Due to lack of existing references, we cannot compare the values to identify the compounds. Further studies have to be done for the identification of the compounds using higher techniques. The BAG_{20} showed significant properties; hence it has been evaluated for antibacterial activity against hospital pathogens.

Praveen *et al.* (2010) investigated the antibacterial activity of four arishtas, namely; Chandanasava, Vidangarishta, Kanakasava and Pippalyasava against *S. aureus* and *E. coli. S. aureus*are more susceptible than *E. coli*. Among the formulation evaluated, Vidangarishta showed higher inhibition of *E. coli* followed by Kanakasava, Chandanasava and Pippalyasava. Vidangarishta had higher inhibition of *S. aureus* followed by Kanakasava, Chandanasava and Pipalyasava.

Ayurvedic herbal formulations which are widely used in ayurvedic medicine in treatment of various infectious diseases were investigated for antibacterial efficacy by Tambekar and Dahikar (2010). The antibacterial activity was determined against 10 enteric pathogenic bacteria. The results showed that bilba churna, chandanadi churna, pushyanug churna and amlachurna were effective against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*. These results well correlate with the present study. It is also confirmed that these herbal formulations can be used not only as a health supplement but also for treatment and control of antibacterial infection.

5. Conclusion

The present study concentrates on determining the pharmaceutico analytical study of beejakarishtaon variation in seasons (sandhana kala). In this study, six samples are made in Summer season, autumn season and winter season with 10 and 20 days duration of fermentation. Out of the 6 samples, 3 samples were kept for 10 days fermentation and other 3 for 20 days' fermentation. Fermentation tests and physicochemical analysis were performed. Beejakarishta of 20 days' fermentation in summer season (BAG_{20}) is used for antibacterial analysis. 100 µl of BAG_{20} showed significant antibacterial activity against *E. coli*, *S. aureus* and *K. pneumoniae*. Therefore, the developed Beejakarishta of 20 days' fermentation in summer season can be used for development of antibacterial drugs for treatment of multidrug resistant (MDR) bacterial infection.

Acknowledgements

The authors are grateful to the Deanship of Scientific Research, Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia for its support for this research work.

Conflicts of interest

The authors declare that there are no conflicts of interest relevant to this article.

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Citation **K.P Shamna, A.K. Muraleedharan and Muhammad Musthafa Poyil (2021). Standardization of beejakarishta preparation with respect to seasonal changes and the determination of its antibacterial activity against selected clinical isolates. Ann. Phytomed., 10(1):160-170. http://dx.doi.org/10.21276/ap.2021.10.1.16**