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A CRITICAL REVIEW OF STANDARDIZATION OF AYURVEDIC *ASAVA-ARISHTA* PART - II APPROACHES AND OUTCOME

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ABSTRACT

The therapeutic attributes of Ayurvedic classical dosage forms *asavas-arishta*, the liquid dosage forms based on self-generated alcohol with their quicker absorption, long shelf life and better compliance with consumers have led to continuous increase in its demand. Many Ayurvedic production units appear to have adopted newer fermentation techniques and manufacturing technologies. These innovations in production, processing and storage underline the importance of the standardization of such products. It is therefore of interest to review the present status of production and standardization of this dosage form in terms of the process and quality and efficacy of the end product. Apart from an attempt to provide quality and standardization parameters, the review provides an analysis and deliberates on significance of changes made to the traditional methods of preparation, ingredients and material used in the process and possible impact on its effectiveness.

KEYWORDS: *Asava–Arishta*, Standardization, Medicinal Wines, QC of Ayurvedic Products, Traditional Pharmacy.

1. INTRODUCTION

Asava-arishta are classical liquid dosage forms wherein infusion or decoction of natural ingredients are fermented with self-generated alcohol based on *sandhana kalpana* as described in Ayurveda.[1] Fermentation is a process of preparing formulations wherein the

therapeutic attributes of a group of ingredients are extracted out of either *swarasa*, freshly extracted juice of plants or *kwatha*, decoction prepared in water with the help of biochemical or microbial fermentation and anaerobic respiration into the liquid.[2] *Asava- arishta* are preferred and prescribed in treatment of a wide range of ailments for its easy to administer form, effectiveness and long term utility. In 'A Critical Review of Standardization of Ayurvedic *Asava-Arishta*' Part – I, Review and Status' we have provided elaborate information from Ayurvedic classical and few modern texts; its comparative and critical study to understand the principles applied in the processes followed for the preparations of *asava-arishta.* In this paper a review of approaches applied for standardization of *asava-arishta* preparations and its outcome are presented in categorical manner and discussed in detail.

2. APPROACHES TO STANDARDIZATION

Standardization of Ayurvedic products is an area of scientific and industrial interest. Large scale production need changes in preparations of classical Ayurvedic products. Satisfying needs of large scale production while adhering to principles of Ayurveda require careful considerations before adapting to new methods. Different parameters have been applied to standardize this self-generated alcohol based liquid classical dosage forms.

Over a period of several years different approaches to standardize *asava-arishta* have been undertaken. These quality control approaches can be broadly divided into three categories -

2.1 Approach related to raw material and equipment

2.2 Approach related to standardization of manufacturing process

2.3 Approach related to standardization of properties and quality of the end product

2.1. Approach related to raw material and equipment

The quality of raw material, herbs and other ingredients used for these preparations have a strong bearing on the process and the finished product. Raw material for these preparations must be authenticated and examined for required quality. Testing of limits of heavy metals, microbial load and residual pesticides are envisaged as these will have impact on the main fermentation process and certain impurities may get retained through the process. It is desirable that the right storage conditions are followed for these raw material before being taken up for main production process.[3] The type of equipment used, material used for fermentation and storage vessels, treatment mooted to the vessels, temperature and storage conditions factors that will impact the process.

2.2. Approach related to standardization of manufacturing processes

The three most relevant parameters for the standardization of *asava - arishta* are -

2.2.1. Effect of temperature

2.2.2. Fermentation time

2.2.3. Use of various vessels and fermentation conditions

2.2.1. Effect of temperature

Temperature affects the process of fermentation. Studies were carried out to understand the role of temperature and the timing of addition of sugar ingredients to the formulation indicate the following results.

A study was carried out on *Draksharishta*, wherein the first batch was prepared by adding jaggery to the *kashaya*, the decoction of herbal ingredients and stirred and boiled for 2 minutes. In another batch the jaggery was added only when the decoction was cooled down to about 40°C and then transferred to procelain jar and kept for fermentation.

Results showed that specific gravity, total solids and total sugars were lesser in the cold *arishta* than hot *arishta*. Cold *arishta* recorded an alcohol generated content of 7.64% whereas in the hot *arishta* there was no alcohol on the day of filteration. Tannin contents were same in both. Lower pH values and higher titratable acidity were observed in *arishta* prepared from decoction with heat than the cold one from fresh juice. It is reasoned that in the hot *Kashaya* formulation, the yeast cells were destroyed because of higher temperature; hence it was not favorable for fermentation process. In cold *Kashaya* the yeast cells were not destroyed and hence it was good for fermentation process.[4,5]

2.2.2. Fermentation time

Effect of keeping the *arishta* over long periods was analyzed for *Amritarishta* for one year. Results showed that the specific gravity, total solid content and sugar content gradually fall with increasing time. There was corresponding increase in the alcohol content, recording maximum in six months. There was no variation in pH value.[6]

A study to find onset of fermentation process and the end of the fermentation process was undertaken. In *Drakshasava* it started on 5th day and completed on 25th day.[5,7] During autumn and summer seasons, it was observed that fermentation takes place in 6 days, in winter it takes 10 days and in rainy and spring seasons it takes 8 days. In the usual practice,

7-10 days are enough in the hot tropical climate and long period of 30 days is allowed in cool temperature climate where biological activity is at its low.[8]

2.2.3. Use of various vessels and preparation conditions[9]

Ayurvedic texts mention use of earthen pot and several others vessels; even of using gold vessel for fermentation process as in the preparation of *Saraswatharishta*.[10] Materials like glass, aluminum, tinned-copper, stainless steel, porcelain jars and earthen pots were used for different preparations of *asava - arishta* where following results were observed.

A study conducted for comparing the use of different vessels in preparation of *Amritarishta,* indicated no change in analytical values of *arishtas* obtained from decoction prepared in different material vessels. However, the decoction prepared in aluminum vessel showed presence of traces of aluminum. This study also revealed that vessels of tinned-copper were a better choice for fermentation process.[11]

Experimental preparation of *Draksharishta* [12] and *Drakshasava*[13] in glass vessels and earthen pots showed no significant difference in quantum of alcohol production. Preparations in glass vessels were found to be more acidic than those from earthen pot. No change was observed in TLC pattern and analytical values. Earthen pot showed more evaporation of water resulting in limited solubility of compounds. This altered pH affected the performance of organisms.[5] According to Indian Pharmacopoeia sterilization can be done by physical, chemical, gas and radiation of vessels.

2.3. Approach related to standardization of properties and quality of the end product:

2.3.1. Physicochemical parameters

2.3.2. Analytical studies

2.3.3. Microbiological studies

2.3.1. Physicochemical Parameters

Physicochemical properties like total solid content, specific gravity, pH, density, extractive values, viscosity, surface tension, refractive index and phytochemical parameters like tannins, alkaloids, reducing sugars, non-reducing sugars, alcohol and total sugar are commonly used parameters for standardization of *asava - arishta*. Some researchers have also tried to analyze iron, magnesium, calcium, phosphate, sulphates, ash value, sodium and potassium contents in the *asava - arishta* formulations.[14,15,16,17]

In the study done on *Kumariasava* significant variation was seen in pH value, extractive value, ash content, specific gravity, reducing sugar, potassium and sodium content of laboratory sample and the market samples. Variation were observed in phytochemical constituents and in experimental studies on animals the different pharmaceutical companies samples exhibited less efficacy as compared to the standard preparation.[18]

Samples of *Ashwagandharishta* and *Aravindasava* of 20 different brands were collected and stored in sealed bottles under room temperature. These were randomly collected and tested for percentage of total alcohol, ethanol, pH and acid value. Percentage of alcohol in each brand of *Ashwagandharishta* was different varying from high of 13.13% to lowest of 7.27%. The pH value of was measured at the time of opening of bottles, on 7th day and 14th day after opening the bottle. *Ashwagandarishta* and *Aravindasava* had weak acidic properties.[14]

2.3.2. Analytical studies of *Asava-Arishta*

Thin Layer Chromatography (TLC) technique is used to test *asava - arishta*. Studies have also been conducted for quantitative analysis of nitrogen content, proteins and lipids as additional test parameters.[13,19]

A TLC study concluded that the standard *Kutajarishta* sample contained *kurchi* alkaloids and the alkaloids isolated from the sample exhibited at least five Dragendroff's staining spots on a chromatogram when developed with appropriate solvent system (Chloroform – Methanol – Ammonia solution; 16:8:0.1).[18] The TLC of *Kharjoorasava* revealed close similarities between *Dhataki* (*Woodfordia fruticosa*) and *Hapusha* (*Juniperus communis L.*) flowers used to trigger fermentation.[20,21]

TLC studies of *Drakshasava* as per pharmacopoeia were carried out using silica gel GF-254 as stationary phase and chloroform: ethylacetate (75:25) as mobile phase. Spots were observed under ultra-violet and visible light. The R_f values of the components were recorded as 0.94, 0.62, 0.46 for hydro-alcoholic extract, for benzene extract 0.98, 0.38, 0.32 and chloroform extract 0.96. The market sample of *Drakshasava* was passed required pharmacopoeia tests.[22]

A simple, precise and accurate HPTLC method was established for the determination of quercetin and rutin in three test formulations of *Draksharishta;* one prepared traditionally, other as per modern methods and the third one, a market sample. The amount of quercetin

was found to be 0.00121, 0.00113 and 0.00109% w/w respectively, while rutin was found to be 0.00262, 0.00224 and 0.00243% w/w respectively.[23]

Few brands of *Kanakasava* were evaluated as per WHO guidelines. It indicated weak acidic properties of *Kanakasava.* Ethanol content of *Kanakasava* was measured by i) specific gravity and ii) gas chromatography methods. Ethanol content from both methods had comparable values within the labeled claims. This study concluded that levels of alcohol, acidity and pH in commercially available *Kanakasava* could be used to establish and formulate procedures for standardization and quality control of *asava-arishta*.[24]

Gas chromatography is a rapid method for the determination of volatile compounds. *Ashwagandharishta* and *Saraswatharishta* were analyzed by gas chromatographic method for the determination of non-ethanol volatile compounds. From a standard mixture, 18 non-ethanol volatile compounds were separated within 15 minutes.[25]

2.3.3. Microbiological Studies

The ingredients like *Woodfordia fruticosa, Madhuka* (*Madhuca longifolia*) flowers, yeast cells, jaggery, honey and direct alcohol are used for the fermentation process in *asava - arishta*.

A comparative study on effect of addition of yeast (*Saccharomyces cerevisiae*) and *W. fruticosa* as a fermenting media of *Kutajarishta* with yeast exhibited that the fermentation process started on the second day and completed within one month whereas with *W. fruticosa* the fermentation started on fifth day and completed in second month. Fermentation may be delayed because of slow natural growth with flowers as against faster multiplication of yeast cells.[26]

In a study on *Kumariasava* two fungi were isolated from *W. fruticosa* flowers identified as *Rhizopus nigridans Ehrenberg* and *Aspergillus niger Van Tieghem*. But these organisms were found to be incapable of fermenting sugar in presence of favorable conditions. In further study *W. fruticosa* flowers were added to sterilized mixture of ingredients of *Kumariasava* including jaggery and kept for one month. No alcoholic fermentation was observed in the preparation. But alcohol was produced in the regular preparation irrespective of whether *W. fruticosa* flowers were added or not and rule out the possibility of *W. fruticosa* being the only source of organism.[27]

W. fruticosa flowers in *Nimba-Arishta* fermentation reported that the flowers contribute to the formulation by releasing enzymes like invertase but not by yeasts.[28] Fermentation triggered with *W. fruticosa* flowers was slower primarily due to meager availability of active yeast cells from nectoriferous region, made available to serve as inoculums. In fact, it took about a week to build an effective population of cells enough to begin fermentation. This lag may also be accounted for the anti-invertase compounds present in *W. fruticosa* flowers that may contribute to slower fermentation in formulation.

W. fruticosa flowers contain wild yeasts, which can tolerate high sugar concentration and are clearly able to bring out the fermentation process in *asava-arishta*. It is also evident that the ancient wisdom to start fermentation with higher sugar concentration and use of osmophilic yeast proved to be the excellent way to prepare *asava-arishta* formulation. In another investigation, it was clearly demonstrated that inoculum of these yeasts can appreciably reduce the time required for fermentation without compromise on quality parameters as well as their biological activity.[5]

3. OUTCOME OF THE STANDARDIZATION EFFORTS

Table – 1 **Outcome of Standardization: Summary Chart**

Parameter	Outcome / Impact	Explanation / Remarks
***1 (a)* Raw material standardization**		
Raw material	Authentication and storage	As per Pharmacopoeia and GMP guidelines
1 (b) Manufacturing processes		
Temperature	Hot decoction: Lower pH & higher (titrable) acidity than cold decoction	Optimum Temperature for Fermentation process is in between 20-35°C
	Hot decoction: Yeast cells are destroyed because of higher temperature; Not favorable for fermentation	
	Cold decoction: Yeast cells are not destroyed hence favorable for fermentation.	
Fermentation time	Increase in alcohol content with increase in time for fermentation.	Fermentation time depends on geographic location and season & ingredients used (Liquid ingredients)
Earthen pot	There is more evaporation of water, limits solubility of compound, alters pH medium and affects performance of micro organisms	Requires delicate handling, tendency of breakage & leakage
Aluminium	Traces of aluminium and ferrous ions found in final product	Inappropriate for production
Wooden vessel	Final Product: Denser in consistency	Absorption of liquid by wood
Stainless steel	No significant variations in physicochemical parameters	Can be used for large scale production

Glass vessels	Final product in glass container is more acidic than in earthen pot	Not convenient for large scale production
Tinned copper	A better choice for fermentation	Can be used for large scale production
***1(c)* End product standardization**		
pH	Affected by temperature and fermentation time	Affect the solubility, stability and quality of the product Essential if the product is more acidic or alkaline
	Utilization of a buffer to control potential changes in the solution pH	
Specific gravity	Temperature	Affect the flow property
Total solid content	Total solid content: Fermentation Time	Solid contents are converted to fermentation product
Reducing sugar percentage (RSP)	RSP reduces with fermentation time	When the percentage remains stable, it is a marker to determine completion of fermentation process
Non-reducing sugar percentage (NRSP)	NRSP varies with temperature and with fermentation time (Due to presence of microorganism)	When the % remains stable, it is a marker to determine completion of fermentation process
Total sugar percent	Total percentage of sugar at fermentation time	Also depends on type of sweetening agent added, Converted to alcohol
	Is less in finished product and varies with type of vessels used.	
Ash value	More in market sample than lab method	Indicative of adulteration
Alcohol percentage	Increased with reference to time duration for fermentation	Important with respect to therapeutic activity and stability
	When prepared in glass vessel	Product may become acidic
Thin layer chromatography	Identification of Phytoconstituents: as a Standard to compare	Qualitative Standardization technique
High performance liquid chromatography	Comparison with marker compound, isolation of functional group used as standard parameter	Quantitative Standardization technique
Woodfordia fruticosa	Two fungi isolated: found to be incapable of fermentation of sugar in presence of favorable condition *W. fucticosa* Flowers: Release enzyme invertase	Invertase catalyzes the hydrolysis of sucrose.
	Bacillus species: Useful for fermentation. Non-alcohol producing organisms that may be due to geographical variation in flowers from different parts	
Madhuca longifolia	Responsible for fermentation	Lower alcohol producing capacity

4. DISCUSSION

An attempt has been made to review and understand the *asava - arishta*, a dosage form from the classical, pharmaceutical (mentioned in part I) and standardization perspective. A review

of the available literature suggests that the industrial approach for the preparation of fermented preparations, *asava–arishta* are based on traditional principles. The new approaches with new techniques contribute to better quality control for the large scale production.

It must *however* be noted that the *asava-arishta* preparations produced adopting modern parameters of standardization as against by traditional method may pose some limitations. The limitations in case of *asava-arishta* standardization arise at all three steps of raw material standardization, process standardization and finished product standardization.

In case of raw material*:* Relevance of *madhura dravyas* i.e. sweeteners like sugar, honey or jaggery to a particular ingredient or group of ingredients; proportion to which it is used and time of addition will definitely affect the alcoholic extraction of therapeutic attributes during fermentation. These are required to be critically and comparatively studied.

W. fruticosa, M. longifolia, Surabeeja or Kinva are added in liquid for fermentation. Among these the most beneficial trigger medium for fermentation is to be examined. Use of yeast as a reliable medium to trigger and enhance fermentation process is known; however, its comparative effect as against *Dhataki* flowers are yet to be established for quality and efficacy.

Timing of additives, at what stage to add, is also important. This is relevant to ingredients and the environmental conditions under which the fermentation process is undertaken. Equally important is the method for addition of additives; mixing, spreading or *pottali* in liquid ingredients should be deliberate.

These different factors will impact the clinical efficacy.

In case of process standardization: Ancient method avoid direct exposure to sunlight to maintain constant temperature; but other factor like humidity should also be studied. Artificial maintenance of temperature and its effect on the properties of the finished product is yet to be studied.

In case of finished product standardization: For different formulations the period of maturation varies. There is a need to evaluate the need for addition of preservative and other effects, if any.

The Central Council for Research in Ayurveda and Siddha (CCRAS) and Pharmacopoeial laboratory for Indian medicine have notified standard protocol for quality control of *asava-arishta*. The compliance of pharmacopoeia standards for compound formulations does help in achieving uniformity and consistency in commercial production of Ayurvedic drugs.

The limitations which arise for the standardization of classical dosage form can also be looked upon as a scope for newer research for a better understanding of the principles and processes, better end product with improved clinical efficacy. To bridge the gap between traditional concepts and modern parameters it is desirable to find solutions with deeper understanding of principles for the use of modern technologies.

5. CONCLUSION

Medicinal wines or *asava-arishta* is a formulation wherein microbial transformation helps in initiating the process of generating alcohol which helps in extracting the attributes and enhancing the bioavailability of the ingredients. Changes in fermentation techniques and adaption to modern technologies are followed for better standardization and quality control. A range of galvanometric, spectroscopic and chromatographic techniques as with TLC, HPTLC or Gas chromatography methods have been applied to evolve standards for *asava–arishta*.

The outcome of these different methods have been variable. Some of these techniques have further potential to contribute to evolve better standardization methods for this liquid dosage form in its totality. There are not many comparative analytical studies between traditional and modern methods of preparations. Confirmation of therapeutic and clinical assessment between the traditional and modern methods of preparations will definitely provide better insights to develop more reliable methods of preparations and better parameters of standardization. Critical evaluation of Ayurvedic principles will help examine innovative applications of present day technologies to develop better standardized, more safe and more clinically effective *asava and arishta*.

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