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PHARMACEUTICAL EVALUATION OF KUMARIASAV

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ABSTRACT

Objective: The present study was aimed to evaluate two batches of classical polyherbal formulation. Kumariasav with respect to their physicochemical (pH, specific gravity, total solid content, reducing and non-reducing sugar content, ethanol content), microbial parameters. It also aimed to evaluate quantitative determination of major heavy metal content in Kumariasav by Inductively Coupled Plasma- Mass Spectroscopy (ICP-MS). **Method:** The analytical methods of evaluation studies were performed as per guidelines of Ayurvedic Pharmacopoeia of India (API). **Results:** The results of standardisation tests obtained were compared with specifications mentioned in monograph of Ayurvedic Pharmacopoeia of India and comparative data of each batch of kumariasav formulation was generated for all quality control parameters performed. **Conclusion:** The results of this research work will serve as effective quality tool for routine quality control analysis of classical polyherbal formulation, Kumariasav.

KEYWORDS: Kumariasav, ICP-MS, physicochemical parameters, Pharmacopoeial standards.

INTRODUCTION

Asava-arishta is a potent dosage form derived from medicinal plants by the process of fermentation. Asavas are prepared usually by fermentation of swaras (expressed juice) while Arishtas are prepared by fermenting the kwaath (decoction). Asava-Arishta are quickly absorbed in the body and are quicker in action, have a long shelf life and are palatable.

Fermentation probably results into transformation of several phytochemical compounds present in medicinal plants, thereby rendering them less toxic and more potent, besides helping in their absorption.[3] Asava-arishta formulations should be evaluated for their physical and chemical properties like viscosity density, refractive index, acid value, alcohol content, reducing and non-reducing sugars, pH etc and are compared with the standards given in the Ayurvedic Pharmacopoeia of India(API) to maintain the quality, safety and efficacy of the product.

In today's modern world, the major drawback faced by Ayurvedic medicines is the unavailability of rigid quality control profiles. It has, therefore, become a need for the manufacturer, pharmacist and physicians to standardise the medicines to maintain batch to batch consistency and efficacy and to achieve highest level of safety. To assure a consistent and acceptable quality product in market, right care should be taken from identification and authentication of raw materials upto the standardisation and testing of the final product which is to be realised in the market.

Ayurveda classical texts have three different references of Kumariasav. For the present study, Kumariasav mentioned in Yogratnakar in Gulma Chikitsa adhyaya has been opted for. Kumariasav is an important asava formulation used in the treatment of Gulma, kasa, Shwaas, piles (arsh), epilepsy (Apasmar) and neurological disorders.

The present study deals with physicochemical analysis as well as organoleptic analysis of two samples of Kumariasav from two different batches.

AIMS

To analyse two samples of Kumariasav for their physicochemical properties.

OBJECTIVES

1. To prepare two different batches of Kumariasav.
2. To analyse Kumariasav with respect to their organoleptic and physicochemical parameters.

MATERIALS AND METHODS

Materials

Ingredients	Instruments	Others
Kumari	Sandhan patra	Fuel
Haritaki	Spoons	Water
Jaggery	utensils	Cloth
Madhu		
Dhataki pushpa		
Loha bhasma		
Tamra bhasma		
Prakshep dravyas (jatiphal, lavang, kankola, jatamansi, chavya, eranda, karkatshringi, bibhitak)		

Methods

The methodology of the present study involves two stages –

1. The preparation of Kumariasav of two different batches.
2. Evaluation of samples of Kumariasav.

[1] Preparation of Kumariasav[1]

Two different batches of Kumariasav were prepared in Department of Rasashastra and Bhaishajya Kalpana, R. A. Podar Medical College, Worli, Mumbai. The procedure was adopted from Yogratnakar, Gulma Chikitsa adhyaya. All the samples were stored in a dark and dry place and were collected for experimentation under aspetic conditions.

- Haritaki kwaath was prepared by 1:16 ratio and reduced to 1/8th.
- This kwaath was filtered and added to a clean and sterile (by dhupan vidhi) container.
- To this, Kumari swaras and jaggery were added.
- The prakshep dravyas, Loha bhasma, Tamra bhasma, Dhataki pushpa and madhu were taken in required quantity and added to the mixture of Haritaki kwaath and Kumari swaras and mixed well and the mouth of the container was sealed.
- The container was kept in a dry place and was periodically checked for the signs of completion of fermentation process.
- The fermented liquid was then filtered and packed in air tight containers.

[2] Evaluation of Kumariasav

Both the samples of Kumariasav were examined for their organoleptic as well as physicochemical properties.

a) Organoleptic properties

The Kumariasav samples were evaluated for their colour, taste and odour as per the procedure mentioned in the Ayurvedic Pharmacopoeia of India.

b) Physicochemical properties

The two in-house preparations of Kumariasav were evaluated by determining their physicochemical properties – pH, viscosity, total solid content, alcohol content, reducing and non-reducing sugars, total phenol content, total microbial load and heavy metal content.

Determination of pH[9]

The digital pH meter was used for the pH measurement after caliberation with buffer solution. pH was noted for both the samples.

Determination of specific gravity

The specific gravity of both the samples was obtained by dividing the weight of the liquid contained in the pycnometer by the weight of water contained determined at 25 degree Celsius.

Determination of Viscosity

Both the samples of Kumariasav under test were filled individually in a U-tube viscometer (Ostwald's viscometer) in accordance with the expected viscosity of the liquid so that the fluid level stands within 0.2 mm of the filling mark of the viscometer. When the capillary is vertical and the specified temperature is attained by the test liquid.

Determination of total solid content[3]

25ml of each sample was taken in separate petri dishes which were previously weighed and allowed to evaporate in an oven so that only the solid part remains in the dish and the remaining liquid part would evaporate. The sample was then weighed and the solid content of the formulation was weighed.

Determination of Ethanol content[3]

Sample (25ml) was transferred to the distillation flask and it's temperature was noted. It was diluted with 150ml of water and distilled. The distilled sample, about 2ml less than the total volume was collected. Water was added to make up exactly same volume of the original test liquid and adjusted to temperature noted before. Specific gravity of this liquid was

determined and alcohol content was analysed using relative density table given in United States Pharmacopoeia.

Determination of reducing and non reducing sugars[4]

20ml of Kumariasav sample was taken and neutralised with sodium hydroxide and evaporated the solution to half volume on waterbath at 50 degree Celsius to remove alcohol. After cooling, zinc acetate, glacial acetic acid and potassium ferrocyanide is added, to which distilled water is added to make a volume of 100ml. Then 10ml of Fehling's solution was added from the burette dropwise and heated to boiling over hot plate till blue colour appeared. Two drops of methylene blue as an indicator was added and the titration was carried till brick red colour was obtained.

Determination of total phenol content[4]

The standard Gallic acid (5mg/ml) solution was prepared in distilled water and prepared the concentration of 100, 200, 300, 400, 500, 600, 700µg/ml for the effective range of the assay. The 1ml of standard Gallic acid solution from each solution from each dilution was taken in 25ml volumetric flask. To it, 10ml water, 1.5ml Follin-Ciocalteau reagent (1N) was added and allowed to stand for 10 minutes. Then 4ml of sodium carbonate (20%) solution was added in each volumetric flask and the final volume was adjusted with distilled water. Readings were taken after 1 hour at 765nm at UV spectrophotometer (Shimadzu 1800) against reagent blank. The calibration curve of absorbance versus concentration was plotted.

Then 1ml of Kumariasav preparation was transferred in 25ml volumetric flask; similar procedure was adopted as above described in preparation of calibration curve. With the help of calibration curve, the phenolic concentration of Kumariasav was determined.

Determination of total microbial load[8]

In a petri dish, a mixture of 1ml of pretreated preparation and about 15ml of liquefied casein soyabean digest agar at not more than 45 degrees is prepared. Alternatively spread the pretreated preparation on the surface of the solidified medium in a petri dish of the same diameter. If necessary, dilute the pretreated preparation so that a colony count of not more than 300 may be expected. Prepare atleast two such petri dishes using the same dilution and incubate at 30 degrees to 35 degrees for 5 days, unless a more reliable count is obtained in the shorter time. Count the number of colonies that are formed. Calculate the results using plates with the greatest number of colonies but taking 300 colonies per plate as the maximum consistent with good evaluation.

Heavy metal analysis using ICP-MS

Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) is a relatively new method for determining multi-element analysis. ICP-MS technology is based on the principles of Atomic Emission Spectroscopy. In high temperature argon plasma, elements present in the sample are dissociated into positively charged ions and detected based on their mass to charge ratios as they subsequently pass through a mass spectrometer. In principle, ICP-MS consists of the following steps: sample preparation and introduction; aerosol generation; ionization by an argon plasma source; mass discrimination and identification by the detection system including data analysis.

ICP-MS is used for detecting inorganic impurities in pharmaceuticals and their ingredients.

- 5ml of the internal standard (deionized water with nitric or hydrochloric acid and Indium and/or Gallium) is added to a test tube along with 10-500 microlitres of Kumariasav.
- This mixture is then vortexed for several seconds or until mixed well and then loaded onto the autosampler tray.
- The sample solution is introduced into the device by means of a peristaltic pump. There it becomes nebulised in a spray chamber.
- The resulting aerosol is injected into an argon-plasma that has a temperature of 6000-8000K.
- Inside the plasma torch, solution is removed from the sample and also atomisation and ionisation occur.
- Only a small amount, part of the ions produced in the plasma further penetrate to the mass spectrometer part.

RESULTS

Table 1: Organoleptic evaluation.

Formulation	Odour	Colour	Taste
Sample 1	Alcoholic	Dark Brown	Sweet
Sample 2	Alcoholic	Dark Brown	Sweet

Table 2: Physicochemical evaluation.

Parameter	Sample 1	Sample 2	Standard values (as per API)[2]
pH	3.7	3.6	3.5 to 4.2
Specific gravity	1.09	1.07	1.01 to 1.10
Viscosity	115 seconds	120 seconds	
Total solids	27.84%	25.50%	NLT 13% w/v
Alcohol Content	5.45%	6.05%	5 to 10% v/v
Reducing sugar	6.67%	6.98%	NLT 7.5% w/v
Non reducing sugar	0.31%	0.26%	NMT 0.30% w/v
Total phenol content	0.061% w/v	0.063% w/v	0.061 to 0.079% w/v equivalent to tannic acid
Total microbial load	0.145×10^8cfu/ml	0.9622×10^5cfu/ml	NMT 10^5/ml
Heavy metal content	Fe – 40.475 ppm Cu – 22.481 ppm Zn – 17.735 ppm	Fe – 46.405 ppm Cu – 28.040 ppm Zn – 19.924 ppm	

NLT – Not less than; NMT – Not more than; API – Ayurvedic Pharmacopoeia of India

Fe – Iron; Cu – Copper; Zn –Zinc

DISCUSSION

In the present study, we evaluated two samples from different batches of Kumariasav formulation for their organoleptic and physicochemical properties.

The completion of fermentation process was tested by the siddhi lakshanas mentioned in the classics – continued lightening of the ignited matchstick, absence of froth and absence of any sound from the sandhan patra. The odour, colour and taste of both samples (S1 and S2) are found to be alcoholic, dark brown and sweet respectively marking the completion of fermentation process.

The pH values of both the S1 and S2 were found to be 3.7 and 3.6 respectively which is moderately acidic and was in required range as mentioned in the API. pH determines the survival and growth of micro-organisms during processing, storage and distribution and also impacts flavour, consistency and shelf life. From the obtained pH values, Inference can be made that both S1 and S2 have exhibited the expected flavour and consistency of the liquid. Also, it can be stated that that the growth of micro-organisms is in check and there has been no harm to it during processing and storage.

The specific gravity of both samples (S1 and S2) were found to be 1.09 and 1.07 respectively whose range mentioned in API is 1.01 to1.10. Specific gravity is used to obtain information about concentration of solutions of various materials. For every substance, there is an unique

specific gravity: Specific gravity helps to determine adulteration. On the basis of the results obtained, It can be derived that both samples are free from any adulteration.

The viscosity of S1 and S2 was 115 seconds and 120 seconds respectively. Viscosity affects the rate at which a product travels through a pipe; how long it takes to set or dry and the time it takes to dispense the fluid into packaging. It determines batch to batch consistency. We can say that both samples haven't been affected by temperature changes or during packaging. Also, it's potency is unaffected by any external causes. It affects the flow property of the formulation which affects to patient compliance and stability of the formulation.[6]

As per the API guidelines for Kumariasav, the total solid content should not be less than 13% w/v. The total solid content found in S1 and S2 was found to be 27.84% and 25.50% respectively which is in accordance with API. Total solid content is important for the pharmacokinetic and pharmacodynamic activity of the drug because of the bioavailability.[6] It can be stated as the total solid content specifies the nutrient content of any formulation.

The alcohol content of Kumariasav was found to be 5.45% in S1 and 6.05% in S2, in accordance with API guidelines which mentions alcohol content of Kumariasav to be between 5 to 10% w/v. Alcohol content is measured to determine batch to batch consistency of the asava. Alcohol acts as a solvent extracting outer layer of Kumari, components such as tannin, flavonoids, colour pigments. As the alcohol concentration increases during fermentation, the extraction of outer layer component increases with implications for the absorption of particular drug i.e. Kumariasav. Alcohols are predominantly produced by yeast during yeast fermentation with ethanol being the main alcohol produced. Bacteria which are pathogenic to human body do not survive the harsh environment of asava-arishta. Alcohol is one of the contributing components to this inhospitable environment. It is used to be thought that no bacteria can survive at very high levels of alcoholic preparations in sandhan kalpana. There is increase in alcohol content with increase in time for fermentation. Alcohol content is also important with respect to therapeutic study and stability.[5]

The percentage of reducing sugars in both samples of Kumariasav was found to be 6.67% and 6.98% respectively. And that of non-reducing sugars was found to be 0.31% and 0.26% for S1 and S2 respectively. They are in accordance with the API guidelines as mentioned in table 2. When the percentage of reducing and non-reducing sugars remains stable, it is considered as a marker to determine the completion of fermentation process.[5] Reducing sugars readily

interact with amino acids and give rise to Malliard reaction products which lead to progressive browning and aroma formation. We can, thus, attribute the dark brown colour and alcoholic odour of Kumariasav odour to this physicochemical property. Reducing and non-reducing sugars also play a significant role in carbohydrate digestion. It also determines the quality of the product.

The total phenol content of S1 and S2 was found to be 0.061% w/v and 0.063% w/v respectively. As per the API guidelines, the total phenolic content should be in the range of 0.061% w/v to 0.079% w/v equivalent to tannic acid. Phenols are weak acids and so, pH and phenol content are inversely proportional. There is a positive relationship between antioxidant activity potential and amount of phenolic compounds in any drug formulation. They are vital antioxidants which exhibit scavenging effect on the free radicals. Phenolic compounds are said to contribute in the colour and sensory components such as allievating bitterness. Browning association with oxidation of phenolic compounds has also been given as the cause of cell death in calli formed in in vitro cultures. Thus, phenolic content in Kumariasav contribute to it's aroma, antioxidant nature and scavenging activity.

The microbial load in both samples was found to be 0.145×10^8 cfu/ml ad 0.962×10^5 cfu/ml respectively. This tests for microbes are designed for the estimation of the number of viable aerobic micro-organisms present and for detecting the presence of designated microbial species in pharmaceutical substances. The bacteria mainly found in fermented foods play a predominant role as carriers of various other health benefits.

The heavy metal concentration of S1 and S2 of Kumariasav was determined by ICP-MS method. Umar et al have mentioned the permissible limits for Iron (Fe), Copper (Cu) and Zinc(Zn) as 261 ppm to 1239 ppm, 150 ppm and 50 ppm respectively.[7] The presence of heavy metals in medicines causes it's deposition in particular organs resulting in immunological response producing a variety of symptoms. The presence of Zinc may be because of processing methods adopted for preparation of Loha bhasma.

CONCLUSION

Study of such formulations in current scenario is of immense importance because asava arishta, the self-fermented products can undergo continuous chemical transformation which goes beyond hydro-alcoholic extraction of the suspended materials. This may result in novel naturals with enhanced therapeutic activity.

From the above study, we conclude that variation on different parameters of evaluation of marketed formulation was may be due to reasons like sources of herbs or plants, collection period, method of preparation.

There was a slight variation in two samples of Kumariasav but were under acceptable limits as per the API.

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