STANDARDIZATION OF MARKETED KUMARI ASAVA- A POLYHERBAL AYURVEDIC FORMULATION

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ABSTRACT
The present study was design for standardization of procured sample of marketed kumari asava was by various physico-chemical standards include pH, viscosity, specific gravity, total solid, total alcohol, reducing sugar and non-reducing sugar, phenolic content as well as heavy metal and mineral content. The pH, viscosity, specific gravity, total solid, total alcohol, reducing sugar and non-reducing sugar was found to be 3.62 ± 0.017, 1.6738 ± 0.010 cp, 1.1243 ± 0.014, 34.68 ±1.990 % w/v, 6.18 ± 0.8141 % w/v, 7.00 ± 1.870 % w/v and 0.27 ± 0.010 % w/v respectively. Total phenolic content of kumari asava by folin-coutigue reagent test by comparing with gallic acid as a standard was found to be 0.063 ± 0.001 % w/v. Quantitative determination of major heavy metal and minerals content of kumari asava by Atomic absorption spectrometer (ASS) showed presence of metal such as Lead 4.29 ppm, Aluminum 183 ppm minerals such as Zinc 27 ppm, Copper 12.6 ppm, Irons142 ppm, Nickel 13.61 ppm and Chromium 0.148 ppm. Present study provides quality control standards which may help in authenticity of the drug and to compile suitable information as per pharmacopoeial monograph for the better utility and safe use of this formulation.

Keywords: Kumari asava, Standardization, Ayurvedic formulation, Quality control.

INTRODUCTION
Traditional systems of medicine have been used throughout the world for centuries. Certain ancient system of medicine especially Ayurveda - the holistic system of medicine from India still used extensively, particularly in their country of origin. Ayurvedic formulations play a vital role in management of diseases and it gives satisfactory result in the therapeutics. Nowadays for rationalize the utility of positive and judicious use of herbal formulation, it becomes a prime need to standardize it by various quality control parameters1. Ayurvedic drugs are traded and consumed in significant quantities but the system is not recognized as a medical system in developed countries. Our share in global herbal market is insignificant and is not commensurate to our herbal wealth. The main reason for the prevalence of this situation is the lack of quality control standards for drugs and formulations used in our traditional systems of medicine. Another important lacuna is the non availability of authentic objective data with respect to their safety and efficacy. To remove these lacunae there is an urgent need to frame guidelines for evolving internationally acceptable standards for ayurvedic formulations. Asava is also one of the weak alcoholic ayurvedic liquid dosage form prepared by using infusion of the drug and allowing to undergo fermentation with the help of raw sugar or honey2. For the standardization of ayurvedic liquid dosage form asava, different quality control test are mentioned in standard official pharmacopoeias.

Present study has to focus the standardization of ayurvedic liquid dosage form asava for determining its quality control standard. The main aim of the present study is to determine quality control standard of well known marketed sample of polyherbal kumari asava.

**MATERIALS AND METHODS**

**Procurement of sample**

For the present study, a well marketed polyherbal formulation Kumari asava no.1 was procured from manufacturing unit of Aushadhi Bhavan, Ayurved Seva Sangh, Nashik, Maharashtra.

**Standardization of kumari asava**

**Determination of pH**

The pH of solution provides a useful practical means for the indication of the acidity or alkalinity of a solution. The pH value of a kumari asava preparation was determined by using a suitable pH meter. Firstly the pH meter calibrated by using 1.021 % w/v solution of potassium hydrogen phthalate as a primary standard and then pH of kumari asava was determined.

**Determination of viscosity**

Viscosity is the internal resistance to the flow of fluid. The viscosity of kumari asava was determined by using Ostwald viscometer. The Ostwald viscometer cleaned, dried and clamped in a vertical position. Both bulbs A and B was immersed in a constant temperature bath. Then taken specified volume of distilled water into the bulb A and sucked the liquid into the bulb B just above the mark M, about half of the bulb A still contain the liquid. The time of flow of the liquid level to fall from the mark M to the mark X was determined. A stopwatch was used to determine the time.

**Determination of specific gravity**

A specific gravity is the ratio of specific weight of the material to the specific weight of the distilled water. A specific gravity bottle of 10ml capacity was cleaned, dried and weighed. It is filled up to the mark with water at the required temperature and weighed. The specific gravity bottle was next filled upto the mark with the sample. The specific gravity was determined by dividing weight of the sample expressed in grams by the weight of the water, expressed in grams.

**Determination of total solid content**

The total solid means the residue obtained when the specific amount of the preparation is dried to constant weight under specified conditions. A 10ml specified quantity was placed in a tarred dish and evaporate at a low temperature as possible until the ethanol was removed and heated on a water- bath until the residue apparently dry. The residue then transferred to an oven operating without a fan and dried at 105°C.

**Determination of alcohol content**

Measured 100ml kumari asava sample in a graduated flask at 20°C and transferred to a separator. Then washed the graduated flask with about 25ml of water and the washing was added to the contents of the separator and sufficient powdered sodium chloride was also added to saturate the liquid. Then 100ml of light petroleum (40°C – 60°C) was mixed to the content and vigorously shaken for 2-3 mins. The mixture was allowed to stand for 15- 30 min and run the lower layer into a distillation flask. Washed the light petroleum in the separator by shaking vigorously with about 25ml of sodium chloride solution and allowed to stand and run the washed liquor into the first brine solution. The mixed solutions was made just alkaline with N/1 sodium hydroxide using solid phenolphthalein as indicator and a little pumice powder and 100ml of water was added. After that distill off 90ml and into a distillate 100ml water was added at the same temperature. Then the specific gravity at 20°C was determined and read off the percentage of ethyl alcohol corresponding to the specific gravity.

**Determination of reducing sugars**

20ml of kumari asava was taken and neutralize with NaOH. The neutralize solution was evaporated to half volume on waterbath at 50°C.
removed alcohol. After cooling 10 ml of 21.9 g zinc acetate, 3ml glacial acetic acid followed by 10.6 g potassium ferrocyanide and distilled water was added to make a volume of 100ml. 10ml of Fehling solution was taken and burette solution was added drop wise and heat to boiling over hot plate till blue color appeared. At this time, two drops of methylene blue was added and the titration was carried on till brick red color was obtained⁹.

**Determination of non-reducing sugars**

20ml of kumari asava solution was taken to which distilled water was added and then boiled 30 minutes on a water bath, after that it was cooled down and its pH was brought to 7. Then volume was made 100ml by addition of distilled water. Then 10ml of Fehling solution was added and solution was titrated till blue color appeared. At this time, two drops of methylene blue was added and the titration was carried on till brick red color was obtained⁹.

**Determination of total phenolic content**

The standard gallic acid (5mg/ml) solutions in distilled water was taken to prepare concentrations 0.100, 0.200, 0.300, 0.400, 0.500, 0.600, 0.700 µg/ml, the effective range of the assay. 1ml of standard gallic acid solution from each dilution was taken in 25ml volumetric flask, added 10 ml water, 1.5ml of Folin-Ciocalteu Reagent (1N) and allowed to stand for 10 min. Then 4ml of Sodium carbonate (20%) solution was added in each volumetric flask and final volume was adjusted with distilled water. Readings were taken after 1 hr at 765 nm by U. V. Spectrophotometer (Shimadzu 1800) against reagent blank. The calibration curve of absorbance versus concentration was plotted. 1ml of kumari asava 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 kumari asava was determined⁹, ⁹.

**Quantitative determination of heavy metals and minerals**

Quantitative determination of major heavy metals and minerals of kumari asava were determined by Atomic absorption spectrometer (ASS) (Perkin Elmer-400), using argon as the carrier gas and flow rate was kept as 1ml/2min. An accurately weighed 5 ml of kumari asava was taken in round bottom flask. 5 ml of concentrated nitric acid was added and refluxed for half an hour on a hot plate at 60-80°C. It was then cooled, 5 ml of concentrated nitric acid was added and warmed on water bath. 2 ml of 30% hydrogen peroxide solution was added to the above mixture and warmed till clear solution was obtained. It was then cooled and filtered through Whatmann-42 filter paper, diluted with deionized water and made up to 100 ml in volumetric flask ¹⁰, ¹¹.

**RESULTS**

The results of present study was determined to compile data to standardize kumari asava a marketed polyherbal ayurvedic preparation by using various physico-chemical standardization parameters including analysis of total phenolic, heavy metal and mineral content. Physico-chemical parameters of kumari asava include pH, viscosity, specific gravity, the total alcohol content, total solid content, reducing sugar and non-reducing sugar was found to be 3.62 ± 0.017, 1.6738 ± 0.010 cp, 1.1243 ± 0.014, 34.68 ± 1.990 % w/v, 6.18 ± 0.8141 % w/v, 7.00 ± 1.870 % w/v and 0.27 ± 0.010 % w/v respectively. Total phenolic content of kumari asava by folin-coutigue reagent test by comparing with gallic acid as a standard was found to be 0.063 ± 0.001 % w/v. (Table 1) (Figure 1).

Quantitative determination of major heavy metal and minerals content of kumari asava by Atomic absorption spectrometer (ASS) showed presence of metal such as Lead 4.29 ppm, Aluminum 183 ppm minerals such as Zinc 27 ppm, Copper 12.6 ppm, Magnesium not detected, Irons 142 ppm, Nickel 13.61 ppm and Chromium 0.148 ppm. (Table 2) The results showed various standards of kumari asava a marketed polyherbal ayurvedic preparation which may comply with pharmacopeial standards and its chemical properties and percentage amount of heavy metal and mineral content showed that this formulation comply with safe and effective standards.

**DISCUSSION**

According to World health organization (WHO) guidelines for herbal drugs, standardized herbal products of consistent quality and containing well-defined constituents are used to provide consistent beneficial therapeutic effects. Pharmacological properties of an herbal formulation depend on phytochemical constituents present therein. Phenolic compounds are a large, heterogeneous group of secondary plant metabolites that are widespread in the plant kingdom ¹². Polyphenols are the products of plant metabolism and can range from simple molecules
to highly polymerized compounds. Phenolics display a vast variety of structures; here only flavonoids, tannins and phenolic acids are reviewed. Flavonoids, a subclass of polyphenols, are the most common polyphenolic compounds found in nature and are further divided into several subclasses including flavones, flavonols, isoflavones, anthocyanins, flavanols, and proanthocyanidins. Kumari asava, a marketed polyherbal ayurvedic preparation contains sufficient phenolic content which predicts it’s a prime nature for their effective bioactivity.

CONCLUSION
Present study thus concluded that, this established physical and chemical standards of polyherbal kumari asava could be used as a valuable tool in the routine standardization to check the batch to batch variation. This study also helps to provide quality control standards which may help in authenticity of the drug and to compile suitable information as per pharmacopoeial monograph for the better utility and safe use of this formulation in therapeutics.

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Table 1: Standardization parameters of Kumari asava

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Values ± SD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>pH</td>
<td>3.62 ± 0.017</td>
</tr>
<tr>
<td>2.</td>
<td>Viscosity</td>
<td>1.6738 ± 0.010 cp</td>
</tr>
<tr>
<td>3.</td>
<td>Specific gravity</td>
<td>1.1243 ± 0.014</td>
</tr>
<tr>
<td>4.</td>
<td>Total solid content</td>
<td>34.68 ± 1.990 % w/v</td>
</tr>
<tr>
<td>5.</td>
<td>Total alcohol content</td>
<td>6.18 ± 0.814 % w/v</td>
</tr>
<tr>
<td>6.</td>
<td>Reducing sugars</td>
<td>7.00 ± 1.870 % w/v</td>
</tr>
<tr>
<td>7.</td>
<td>Non-reducing sugars</td>
<td>0.27 ± 0.010 % w/v</td>
</tr>
<tr>
<td>8.</td>
<td>Total phenolic content</td>
<td>0.063 ± 0.001 % w/v</td>
</tr>
</tbody>
</table>

* An average of three determinations

Table 2: Heavy metal and mineral content of Kumari asava

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Heavy metals and Mineral Content</th>
<th>Values (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Lead</td>
<td>4.29</td>
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<tr>
<td>2.</td>
<td>Aluminum</td>
<td>183</td>
</tr>
<tr>
<td>3.</td>
<td>Zinc</td>
<td>27</td>
</tr>
<tr>
<td>4.</td>
<td>Copper</td>
<td>12.6</td>
</tr>
<tr>
<td>5.</td>
<td>Magnesium</td>
<td>N. D.</td>
</tr>
<tr>
<td>6.</td>
<td>Iron</td>
<td>142</td>
</tr>
<tr>
<td>7.</td>
<td>Nickel</td>
<td>3.61</td>
</tr>
<tr>
<td>8.</td>
<td>Chromium</td>
<td>0.148</td>
</tr>
</tbody>
</table>

N. D. - Not Detected

Fig. 1: Calibration curve of standard drug (gallic acid) for determination of total phenolic content (Concentration Vs Absorbance)
REFERENCES