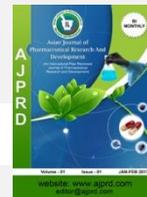


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Research Article

STANDARDIZATION OF JAMBAVASAVA - A POLYHERBAL AYURVEDIC FORMULATION**Chumbhale Deshraj S^{1*}, Chavan M. J.¹, Upasani C. D.²**¹Department of Pharmacognosy, Amrutvahini College of Pharmacy, Sangamner-422 608, Maharashtra, India.²SNJB'S Shriman Sureshdada Jain College of Pharmacy, Chandwad, Nashik – 423 101, Maharashtra, India**ABSTRACT**

Standardization of herbal formulation is an essential tool for assessment of quality of drugs based on physical properties and chemical content. The present paper reports on standardization of procured sample of marketed Jambavasava prescribed for the treatment of diabetes. Present study includes various physico-chemical standards such as pH, specific gravity, viscosity, acid value, total solid, total alcohol, reducing sugar and non-reducing sugar, heavy metal and mineral content. Quantitative chemical estimation of total phenolic content was done by Folin- Ciocalteu reagent method using gallic acid as a standard. Also major heavy metals and minerals content was determined by Atomic absorption spectrometer (AAS). The pH, specific gravity, viscosity, acid value, total solid, total alcohol, reducing sugar and non-reducing sugar was found to be 3.87 ± 0.010 , 1.23 ± 0.006 , 1.663 ± 0.011 cp, 0.08 ± 0.004 , 33.67 ± 0.005 % w/v, 7.86 ± 0.015 % v/v, 8.03 ± 0.058 % w/v and 0.25 ± 0.030 % w/v respectively. Phenolic content of Jambavasava by Folin- Ciocalteu reagent method by comparing with gallic acid as a standard was found to be 0.062 ± 0.001 % w/v. Estimated heavy metals and minerals of Jambavasava showed presence of heavy metals such as Lead 4.96 ppm, Aluminum 182 ppm and minerals includes Zinc 26.90 ppm, Copper 11.6 ppm, Irons 158 ppm, Nickel 3.59 ppm and Chromium 0.29 ppm indicated within a safety limit. Present study has to compile the quality control standards of polyherbal Jambavasava for the authenticity of drug sample and its pharmacopoeial monograph information for the better and safe use in the therapeutics.

Keywords: Jambavasava, Physico-chemical standards, Ayurvedic liquid formulation, Quality control.

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INTRODUCTION

Traditional ayurvedic polyherbal formulations especially liquid dosage forms such as *Asava* and *Arishta* have been used as medicines for over 3000 years to treat various disorders and are also taken as appetizers and stimulants. Due to their medicinal value, sweet taste, and easy availability people consume higher doses of these drugs for longer periods. The huge manufacturing and sale of this preparations covered vital place in ayurvedic pharmaceutical industry. The preparation and sale of 25 varieties of *Asava* and 34 varieties of *Arishta* has been legalised and listed in the official Ayurveda Pharmacopoeia of Sri Lanka (1). *Asava* is a weak alcoholic ayurvedic liquid preparation formulated by using infusion of the drug sample by allowed to undergo fermentation with using raw sugar

or honey (2). Ayurvedic multi-herbs formulation are generally available is a mixture of more than one plant constituents having vital role in management of diseases and it gives satisfactory result in the therapeutics. To develop standardization parameters of polyherbal formulations, it's a prime need to justify the physico-chemical standards of formulation by various quality control tests (3). The official pharmacopoeias mentioned standardization procedures and different quality control test include quantitative parameters for ayurvedic preparations is a milestone to rationalize utility of quality and safe product to the consumer. Present study was undertaken to standardize, *Jambavasava* an ayurvedic formulations which is available commercially to develop

internationally acceptable quality standards and authentic data with respect to their safety and efficacy.

Jambavasava is a well marketed polyherbal ayurvedic formulation contains herbs that plays an important role in the treatment of diabetes and it gives satisfactory result in the therapeutics. It contains extracts of *Jamun* (*Eugenia jambolana*, Myrtaceae), *Neem* (*Azadirachta indica*, Meliaceae), *Methi Beej* (seeds of *Trigonella foenum-graecum*, Apiaceae), *Karela* (*Momordica charantia*, Cucurbitaceae), *Shudha Shilajeet*, *Gudmar* (*Gymnema sylvestre*, Asclepiadaceae), *Triphala* (*Terminalia bellerica*, Combretaceae; *Terminalia chebula*, Combretaceae; *Embllica officinalis*, Euphorbiaceae), *Kutki* (*Picrorhiza kurroa*, Plantaginaceae), *Belpatra* (*Aegle marmelos*, Rutaceae), *Kavach Beej* (*Mucuna pruriens*, Fabaceae), *Haridra* (*Curcuma longa*, Zingiberaceae), *Triwang Bhasma* [Shuddha Naga (Purified Lead 10 g), Shuddha Vanga (Purified Tin 10 g), Shuddha Yashada (Purified Zinc 10 g)], *Dhataki* (*Woodfordia fruticosa*, Lythraceae), *Gokharu* (*Tribulus terrestris*, Zygophyllaceae), *Chirayta* (*Swertia chirata*, Gentianaceae), *Kalijiri* (*Centratherum anthelminticum*, Asteraceae), *Lodhra* (*Symplocos racemosa*, Symplocaceae), *Rasana* (*Pluchea lanceolata*, Asteraceae), *Shatavari* (*Asparagus racemosus*, Asparagaceae), *Sunthi* (*Zinziber officinale*, Zinziberaceae), *Askand* (*Withania somnifera*, Acanthaceae), *Dalchini* (*Cinnamomum zeylanicum*, Lauraceae), *Erandmul* (*Ricinus communis*, Euphorbiaceae), *Punarnava* (*Boerhavia diffusa*, Nyctaginaceae).

Present study has to explore standardization of ayurvedic liquid dosage form especially *Asava* for determining its quality control parameters. The main aim is to find physico-chemical standards of well known marketed sample of polyherbal *Jambavasava*.

MATERIALS AND METHODS

Procurement of sample

For the present study, commercially marketed sample of *Jambavasava* a polyherbal formulation was procured from manufacturing unit of Abhay Ayurvedic Pharmacy, Osmanabad (M. S.), Maharashtra.

Physicochemical Standardization of *Jambavasava*

Determination of pH

The pH of solution is an important practical means for the indication of the acidity or alkalinity of a solution. The pH value of a polyherbal *Jambavasava* preparation was determined by using a pH meter. The prime step was performed to calibrate the pH meter by using 1.021 % w/v solution of potassium hydrogen phthalate as a primary standard. After calibration, the pH values of the commercially available brands of *Jambavasava* was measured at the time of opening the bottle and seven days and 14 days after opening the bottle. During the 14 days, the drug bottles kept at room temperature were shaken well manually to mimic the normal practice during consumption and opened two to three times per day and then pH of *Jambavasava* was determined by official pharmacopeia method (4).

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 10 ml capacity was cleaned, dried and weighed. It was 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 of *Jambavasava*, at the same temperature and weighed. The specific gravity was determined by dividing weight of the sample expressed in grams by the weight of the water, expressed in grams (5).

Determination of viscosity

Viscosity is the internal resistance to the flow of fluid. The viscosity of *Jambavasava* was determined by using Ostwald viscometer. Firstly, clamp the clean and dry Ostwald viscometer in a vertical position, with both bulbs A and B immersed in a constant temperature bath. The specified volume of distilled water was taken into the bulb A and sucks 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. The stopwatch was used to determine the time (6).

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. The specified quantity (10 ml) of sample, *Jambavasava* was taken in a tared dish. The sample evaporates at a low temperature as possible until the ethanol is removed and heat on a water-bath until the residue is apparently dry. Transfer to an oven operating without a fan and dry at 105°C (7).

Determination of total alcohol content

The 100 ml of the sample, *Jambavasava* was measured in a graduated flask at 20°C. Transferred the sample to a separator and wash out the graduated flask with about 25ml of distilled water and added the washing to the contents of the separator, and also added sufficient powdered sodium chloride to saturate the liquid. Afterward, added 100 ml of light petroleum ether (40°C – 60°C) and shaken vigorously for 2-3 mins. Allowed the mixture to stand for 15- 30 mins and run the lower layer into a distillation flask. Wash out the light petroleum in the separator by shaking vigorously with about 25ml of sodium chloride solution and then allowed to stand and run the wash liquor into the first brine solution. The mixed solutions made alkaline with N/1 sodium hydroxide using solid phenolphthalein as indicator and then added a little pumice powder and 100 ml of water. Distilled out the 90 ml and then added into 100 ml distilled water at the same temperature. The specific gravity of distillate was determined at 20°C. The percentage of total alcohol corresponding to the specific gravity was noted (8, 9).

Determination of reducing sugars

The 20 ml of *Jambavasava* was taken and neutralize with sodium hydroxide and evaporated the solution to half volume on waterbath at 50°C to remove alcohol. After cooling added 10 ml of 21.9 g zinc acetate and 3 ml glacial acetic acid and 10.6 g potassium ferrocyanide and

to which distilled water was added to make a volume of 100ml. The 10ml of Fehling solution and from burette solution was added dropwise to the preparation with heat to boiling over hot plate till blue color appeared. Two drops of methylene blue as a indicator was added and the titration was carried till brick red color was obtained (10).

Determination of non- reducing sugars

The 20 ml of *Jambavasava* sample was taken to which distilled water was added and boiled for 30 minutes in a water bath. After that it was cooled down and its pH was brought to 7. Then volume was made 100 ml by addition of distilled water. Then 10 ml 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 obtained (10).

Determination of total phenolic content

The standard gallic acid (5 mg/ml) solutions was prepared in distilled water and prepared the concentrations of 100, 200, 300, 400, 500, 600, 700 µg/ml for the effective range of the assay. The 1 ml of standard gallic acid solution from each dilution was taken in 25 ml volumetric flask, added 10 ml water, 1.5 ml of Folin- Ciocalteu Reagent (1N) and allowed to stand for 10 min. Then 4 ml 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 verses concentration was plotted.

The 1 ml of *Jambavasava* preparation was transferred in 25 ml volumetric flask; similar procedure was adopted as above described in preparation of calibration curve. With the help of calibration curve, the phenolic concentration of *Jambavasava* was determined (10, 11).

Quantitative determination of heavy metals and minerals

Quantitative determination of major heavy metals and minerals of *Jambavasava* 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 *Jambavasava* 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 (11, 12).

RESULTS

Present study was design to standardize *Jambavasava* by using different physico-chemical standardization parameters including analysis of total phenolic, heavy metal and mineral content. Physico-chemical parameters of *Jambavasava* includes the pH, specific gravity, viscosity, acid value, total solid, total alcohol, reducing sugar and non-reducing sugar was found to be $3.87 \pm$

0.010 , 1.23 ± 0.006 , 1.663 ± 0.011 cp, 0.08 ± 0.004 , 33.67 ± 0.005 % w/v, 7.86 ± 0.015 % v/v, 8.03 ± 0.058 % w/v and 0.25 ± 0.030 % w/v respectively. Phenolic content of *Jambavasava* by Folin- Ciocalteu reagent method by comparing with gallic acid as a standard was found to be 0.062 ± 0.001 % w/v. (Table 1) (Figure 1)

Table 1: Standardization parameters of *Jambavasava*

Parameters	* Values \pm SD
pH	3.87 ± 0.010
Specific gravity	1.23 ± 0.006 g/ml
Viscosity	1.663 ± 0.011 cp
Acid Value	0.08 ± 0.004
Total solid content	33.67 ± 0.005 % w/v
Total alcohol content	7.86 ± 0.015 % v/v
Reducing sugars	8.03 ± 0.058 % w/v
Non- reducing sugars	0.25 ± 0.030 % w/v
Total phenolic	0.062 ± 0.001 % w/v

*An average of three determinations

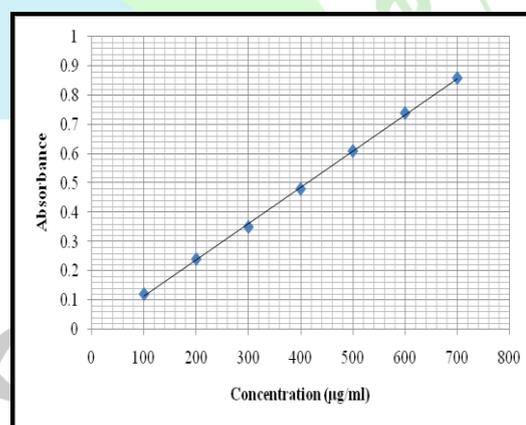


Fig. 1: Total phenolic content determination by calibration curve of standard drug (gallic acid)

Quantitative determination of major heavy metal and minerals content of *Jambavasava* by Atomic absorption spectrometer (ASS) showed presence of metal such as Lead 4.96 ppm, Aluminum 182 ppm and minerals includes Zinc 26.90 ppm, Copper 11.6 ppm, Irons 158 ppm, Nickel 3.59 ppm and Chromium 0.29 ppm. (Table 2)

Table 2: Heavy metal and mineral content of *Jambavasava*

Heavy metals and Mineral Content	Values (ppm)
Lead	4.96
Aluminum	182
Magnesium	N. D.
Zinc	26.90
Copper	11.6
Iron	158
Nickel	3.59
Chromium	0.29

N. D. - Not Detected

DISCUSSION

According to World health organization (WHO), standardized herbal products of consistent quality and containing well-defined constituents are used to provide consistent beneficial therapeutic effects. Present study was established physical and chemical standards of polyherbal *Jambavasava* could be used as a valuable tool in the routine standardization to check the batch to batch variation. The sample was tested for pH, and acid value percentage of total alcohol (v/v) content. Especially, acid value of sample indicates the presence of total free acids present in the product. Acids are produced during preparation (especially in the fermentation process) and storage (oxidation of alcohols) and are responsible for the sour taste of these preparations (13).

The results highlighted that the levels of alcohol in *Jambavasava* was lower than those in fortified wines and distilled spirits. The fortified wines and distilled spirits contain 18 to 21% and 40 to 50% alcohol respectively. In the commercially available *Jambavasava* the levels of alcohol averagely contains only 7.86 ± 0.015 % v/v of self generated alcohol. It indicates that the alcohol content of *Jambavasava* was understood within a limit.

The results of present study imparts estimation of chemical content of ayurvedic polyherbal formulation, *Jambavasava* by estimation of total phenolic portion which indicated that the number of herbs as a composition of formulation contains phenolic compounds. Phenolic compounds are a large, heterogeneous group of secondary plant metabolites that are widespread in the plant kingdom (14). 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 (15). Present phenolic content indicated the formulation contains amount of phenolic content as a bioactive compound responsible for its therapeutics properties and established conclusion of indicative bioactive marker for the identification and authenticity of formulation.

The results explore chemical analysis especially heavy metal and mineral content analysis which helps to compile monograph parameters as per safety guidelines of World Health Organization (WHO) (16). Contamination of medicinal plant materials with arsenic and heavy metals (like cadmium and lead) can be attributed to many causes, such as environmental pollution, and traces of pesticides. The limits (parts per million) of such heavy metals in medicinal plants should remain within the limits of specifications. The results of commercially available *Jambavasava* showed percentage amount of heavy metal and mineral content showed that this formulation comply with safe and effective standards.

CONCLUSIONS

Present study attempted that, the scientific data of quality control standards which may help in authenticity of the drug and provides the suitable information for compilation of pharmacopoeial monograph for the better utility and safe use of this formulation in therapeutics.

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CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

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