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Standardization and evaluation of marketed Kumari Asava of Baidyanath

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Abstract

Objective: Ayurveda comprise of various type of medicines including the fermented forms. Asavas (fermented infusions) are regarded as valuable therapeutics due to their efficacy and desirable features. Additional benefits are expected, e.g. it provides nourishment to the body. Useful in weight gain treatment, improve taste, useful in anorexia, useful in reliving abdominal pain due to gastritis and digestive tract disorder, ascites, constipation, tuberculosis, urinary tract disorders, diabetes, epilepsy treatment difficulty in urination, use to improve memory, useful in improving quality of semen and sperm, urinary calculi, intestinal worm infestation and bleeding disorders. Standardization of herbal formulation is essential in order to assess the quality, purity, safety and efficacy of the drug.

Methods: The present study deals with the standardization of this formulation by using the parameters like organoleptic characters, physical properties and various physico-chemical properties such as total solid, reducing sugar, non reducing sugar, surface tension, specific gravity, viscosity, boiling point, solubility test, UV examination for absorbance of sample drug, colorimetric analysis, acid value, pH determination and heavy metal content study carried out to ascertain the quality, purity and safety of this herbal formulation.

Results: The formulation of Kumari Asava contains all good characters of an ideal Asava and it was found to be harmless, more effective and economic. The marketed samples have been standardize and evaluated on the basis of the above mentioned parameters which show satisfactory results, but the efficacy of the products can only be judged by doing the pharmacological & clinical methods, which is suggested as future scope of R&D.

Conclusion: The study shows that the contents of formulation presents within the permissible limits as per WHO. The study could be helpful in authentification of Kumari Asava. The result of present study will also serve as reference monograph in the preparation of Asavas.

Keywords: Standardization, Baidyanath Kumari Asava, Physico-chemical parameter

Introduction

An herb is a plant or part of a plant valued for its medicinal, aromatic, or savoury qualities. Herbal medicine or herbalism is the use of herbs or herbal products for their therapeutic or medicinal value. Asava are one of the most popular dosage forms of Ayurvedic medicaments due to their long shelf life, quick action and high therapeutic effectiveness [1]. Traditional medicines are effective but the standardization of Ayurvedic formulations is essential in order to assess the quality of drugs [2]. Ayurvedic preparations achieved paramount importance in contemporary life owing to the safety and efficacy when compared with those of synthetic drugs. But due to lack of proper standardization at each stage from starting to culmination results in inferior quality and less demand [3]. Total global herbal market is of size 62.0 billion dollars, in this India's contribution is only one billion dollars. The forecast is that the global market for herbal products is expected to be $5 Trillion by 2050 [4, 5]. Tyler defines herbal medicines as crude drugs of vegetable origin utilized for treatment of diseases states, often of a chronic nature, or to attain or maintain a condition of improved health. Current demands for herbal medicines have resulted in an annual market of $1.5 billion and increasingly widespread availability [6].

Historically, herbal medicines have played a significant role in the management of both minor and major medical illness [6]. Many common drugs we use today were derived from plant-based sources. For example, scientists originally derived aspirin from willow bark; herbalists prescribe white willow for headaches and pain control [7]. The majority of herbal products available today were originated from the same traditional formulas or ingredients. Herbal medicine is about 70% of Traditional Chinese Medicine going in deeper within the body to treat the root cause [4]. Therefore, herbal remedies would become increasingly important especially in developing countries. India, with its biodiversity has a tremendous potential and advantage in this emerging area [5].

Advantages of herbal medicines:

- Reduced risk of side effects

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- Effectives with chronic conditions
- Lower cost
- Widespread availability [8].
- They have large amount of use.
- The medicinal plants have renewable source of cheaper medicines.
- They have better patient tolerance as well as acceptance.
- Improvements in the quality, efficacy and safety of herbal medicines with the development of science and technology.
- Prolong and apparently uneventful use of herbal medicines may offer testimony of their safety and efficacy.
- Throughout the world herbal medicines have provided many of the most potent medicines to the vast arsenal of drugs available to modern medical science, both in crude form as well as a pure chemical upon which modern medicines are constructed.

Medicinal plants have played a key role in world health. There is a growing focus on the importance of medicinal plants in the traditional health care system (viz. Ayurveda, Yoga, Unani, Siddha & Homoeopathy) in solving health care problems. Systematic approach and well-designed methodologies for the standardization of herbal raw materials and herbal formulations are developed. In view of the growing interest in herbal medicines, methods for standardization of herbal drugs are developed and used in different formulation. Standardization brings important benefits to business including standardization as the code of conduct in order to ensure the consistent efficacy that manufacturers should use to ensure batch-to batch consistency of their products. The quality of herbal drugs is the sum of all factors which contribute directly or indirectly to the safety, effectiveness and acceptability of the product. Due to advancement in the chemical knowledge of crude drugs various methods like botanical, chemical, spectroscopic and biological methods are used for estimating active constituents present in crude drugs [8]. Standardization methods should take into consideration all aspects contributing to the quality of the herbal drugs. Standardization brings important benefits to business including a solid foundation upon which to develop new technologies and an opportunity to share and enhance existing practices. Standardization also play a pivotal role in assisting governments, administrations, regulators and the legal profession as legislation, regulation and policy initiatives are all supported by standardization [6].

Materials and methods

- Introduction of sample
- Sample name: Baidyanath Kumari Asava
- Ingredients: Kumari, Triphla, Trikatu, Akarkara, Chaturjat, Dhania, Devdaru, Pipalmul, Chitrakmool, Punarva, Loha Churna, Daru Haldi, Rasna, Danti Mool, Swarn Makshik Bhasm, Pippal, Dhataki Pushpa, Jaggery (Gud) etc.

The Asava (fermented infusion) are considered as a unique and valuable therapeutics in Ayurveda, due to their medicinal value, sweet taste and easy availability. People are prone to consume higher dosage of these drugs for longer periods. In general, all Asava have 5-10% of alcohol. Though these Ayurvedic medicines contain alcohol, they are quite safe to prescribe and to consume. Kumari Asava is used in the treatment of abdominal distension, respiratory conditions like cough, cold, wheezing, piles vata imbalance diseases and certain neurological condition. Further traditional uses of Kumari Asava are to improve strength, skin complexion and digestion power. Asava are considered unique as they have several advantages. Classical literature indicates that they posses better keeping quality, which is likely due to the contribution of fermentation to preservation. The microbes involved in this process mediate this process; enhanced therapeutic properties, which may be due to the microbial biotransformation of the initial ingredients of Asava into more effective therapeutics as end products; improvement in the extraction of drug molecules from the herbs by alcohol-aqueous milieu, which is also produced by microbes; improvement in drug delivery in the body, which may also be at least partially due to microbial biotransformation either because of biotransformation or because of alcohol-aqueous milieu. Though there is an extensive list of Asava products with diverse medicinal properties, there is lot of scope to work with them microbiologically, Biochemically and pharmacologically. This will make Asava as scientifically validated products for the betterment of human life [9-13].

Figure 1: Baidyanath Kumari Asava

- Development of standardization parameters for Baidyanath Kumari Asava

1. Study of organoleptic characters
   a) Colour
   b) Odour
   c) Taste

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2. Determination of physio-chemical parameters
   a) Total solid content
   b) Reducing sugar
   c) Non reducing sugar
3. Qualitative estimation of selected phyto-constituents
   a) Glycosides
   b) Alkaloids
   c) Flavonoids
   d) Steroids
   e) Amino acids
   f) Carbohydrates
   g) Tannins
   h) Saponins
4. Evaluation of Asava
   a) Surface tension
   b) Specific Gravity
   c) Solubility test
   d) Viscosity
   e) Boiling Point
   f) UV examination for absorbance of drug
   g) Colorimetric analysis
   h) Acid value
   i) UV examination for absorbance of drug
   j) Colorimetric analysis
   k) Acid Value
5. Determination of pH
6. Establishing the safety pertaining to heavy metals

Methods:

1. Study of organoleptic characters

The polyherbal formulation is studied for organoleptic characters like colour, odour and taste using the sensory organs of our body.

2. Determination of physio-chemical parameters

a) 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 10 ml 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 ºC.

b) Determination of reducing sugars

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

c) Determination of non- reducing sugars

20 ml of Kumari Asava solution was taken to which distilled water was added and then boiled 30 min on 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 colour appeared. At this time, two drops of methylene blue was added and the titration was carried on till brick red colour was obtained.

3. Qualitative estimation of selected phyto-constituents

a) Test for *Aloe vera*

- Borntrager's test: Boil the test material with 1 ml of sulphuric acid in a test tube for five minutes. Filter while hot. Cool the filtrate and shake with equal volume of dichloromethane or chloroform. Separate the lower layer of dichloromethane or chloroform and shake it with half of its volume of dilute ammonia. A rose pink to red colour is produced in the ammoniacal layer.
- Modified Borntrager's test: Boil 200 mg of the test material with 2 ml of dilute sulphuric acid. Treat it with 2 ml of 5% aqueous ferric chloride solution (freshly prepared) for 5 min, shake it with equal volume of chloroform and continue the test as above. As some plants contain anthracene aglycone in a reduced form if ferric chloride is used during the extraction, oxidation to anthraquinones takes place, which shows response to Borntrager's test.

b) Test for Loha churna

- Foam test: Shake the drug/sample extract vigorously with water. Persistent foam observed, confirms the presence of saponins.
- Haemolytic test: Add drug/sample extract or dry powder to one drop of blood placed on glass slide. Haemolytic zone appears.

c) Test for alkaloids

- Dragendroff"s test: Alkaloids give reddish brown precipitate with Dragendroff"s reagent (Potassium bismuth iodide solution).
- Mayer's test: Alkaloids give cream colour precipitate with Mayer's reagent (Potassium mercuric iodide solution).

d) Test for flavonoids

- Alkaline reagent test: To the test solution add few drops of solution hydroxide solution, intense yellow colour is formed which turns to colourless on addition of few drops of dilute acid indicate presence of flavonoids.

e) Test for amino acids

- Ninhydrine test: To the test solution add Ninhydrine solution, boil, violet colour indicates presence of amino acids.

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f) Test for steroids

- Salkowski test: Treat the extract with few drops of concentrated sulphuric acid, red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of triterpenoids.

g) Test for carbohydrates

- Fehling test: Take Fehling solution (A+B) in a test tube, add sample solution and boil. Formation of a precipitate of brownish and cuprous oxide, presence of reducing sugar.
- Benedict test: Take sample solution and add Benedict reagent, mix well, boil the mixture vigorously for two minutes. To produce red, yellow or green colour precipitate, presence of reducing sugar.

4. Evaluation of Asava

a) Surface tension measurement

Measurements were carried out with a 10 ml of Asava at room temperature. Thoroughly clean the stalagmometer using chronic acid and purified water, because surface tension is highly affected with grease or other lubricants. The data calculated by following equation given below:

$$R_2 = (W_3\text{-}W_1)\, n_1 \times R_1\, (W_2\text{-}W_1)\, n_2$$

Where W_1 is weight empty beaker, W_2 is weight of beaker with ethanol, W_3 is weight of beaker with Asava, n_1 is no. of drops of ethanol, n_2 is no. of drops of Asava, R_1 is surface tension of ethanol at room temperature, R_2 is surface tension of Asava.

b) Specific gravity

The two methods are commonly used for determination the specific gravity of liquid one method use the hydrometer and instrument that gives a specific gravity reading directly. A second method called a bottle method uses a specific gravity bottle that is a flask makes to hold a known volume of liquid at a specified temperature usually 20 ºC. The bottle is weighted filled with the liquid. Whose specified gravity is to be found and weight again. The different weight is divided by the weight of equal volume of water to give the specific gravity of the liquid.

c) Solubility

The solubility of the sample can be determine by the mixing the sample with in a test tube with the particular chemicals such as water, ethanol, benzene, Ether.

d) 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 were 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.

e) Boiling point

Make a test tube assembly and a water bath assembly. Then reheat the water bath and repeat the cooling process two more times. Record the temperature reading after each trial, and average all three trials. The published boiling point of ethanol is 78.29 ºC. Calculate the error between the observed boiling point and the published value of the boiling point.

f) UV examination for absorbance of drug sample

Obtain of UV examination for absorbance of drug sample may be done by taking the reading of blank sample (ethanol). Then the reading of drug sample (Kumari Asava) was observed. Examine the spectrum for the absorbance of drug sample by comparing the absorbance of peak with the standard peak of drug sample.

g) Colorimetric analysis

Obtain of colorimetric analysis of drug sample may be done by taking the reading of blank sample (ethanol). Then the reading of drug sample (Kumari Asava) was observed. Compare the reading of drug sample between the observed and standard value of drug sample which has been obtain from UV examination of absorbance of drug sample.

h) Acid value

10 g of formulation was dissolved in 50 ml of equal volume of ethanol and ether previously neutralized with 0.1M KOH to phenolphthalein solution. To it 1 ml of phenolphthalein solution was added and titrated with 0.1M KOH until solution remains faint pink after shaking for 30 sec.

5. Determination of pH

pH was determine at the temperature of 27 ºC ± 2 ºC. In the case of Asava, pH was read directly in the sample in the pH meter.

6. Establishing the safety pertaining to heavy metals

Table 1: For Cadmium

Experiment	Observation	Result
NH_4OH add in a sample solution	White ppt is absent	p/a cadmium
Potassium Ferro cyanide is added	White ppt is absent	p/a cadmium

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Table 2: For bismuth

Experiment	Observation	Result
NH_4OH add in a sample solution	White ppt is absent	p/a bismuth
H_2S gas is added	Dark brown ppt is absent	p/a bismuth

Table 3: For lead

Experiment	Observation	Result
Dil. HCL add in a sample solution	White ppt of $CaCl_2$ is absent	p/a lead
KI is added	Yellow ppt is absent	p/a lead

Results and discussion

- Determination of organoleptic characters

Table 4: Organoleptic characters

S. No.	Test	Inference
1	Colour	Brownish
2	Odour	Characteristics
3	Taste	Bitter

- Determination of physico- chemical parameter

Table 5: Total solids

S. No.	Total solids
1	0.1133

Table 6: Reducing sugar

S. No.	Reducing sugar
1	Present

Table 7: Non reducing sugar

S. No.	Non Reducing sugar
1	Present

- Qualitative analysis

Table 8: Qualitative analysis

S.No.	Chemical Constituents	Aqueous extracts
1	Glycosides	Present
2	Alkaloids	Present
3	Flavonoids	Absent
4	Steroids	Absent
5	Amino acid	Present
6	Carbohydrates	Present
7	Tannins	Present
8	Saponins	Present

- Evaluation of Asava

Table 9: Surface tension

S. No.	Surface tension dynes/cm (S.E.M.)
1	1.47 ± 1.74

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Table 10: Specific gravity

S. No.	Density gm/cm^3(S.E.M.)
1	0.48 ± 2.98

Table 11: Solubility

S. No.	Chemicals	Observed
1	Water	Soluble
2	Ethanol	Soluble
3	Benzene	Sparingly soluble
4	Ether	Not soluble

Table 12: Viscosity

S. No.	Viscosity (g cm^{-1} s^{-1}) (S.E.M.)
1	0.0012 ± .35

Table 13: Boiling point

S. No.	Standard value (°C)	Observed value (°C)
1	78.37	76

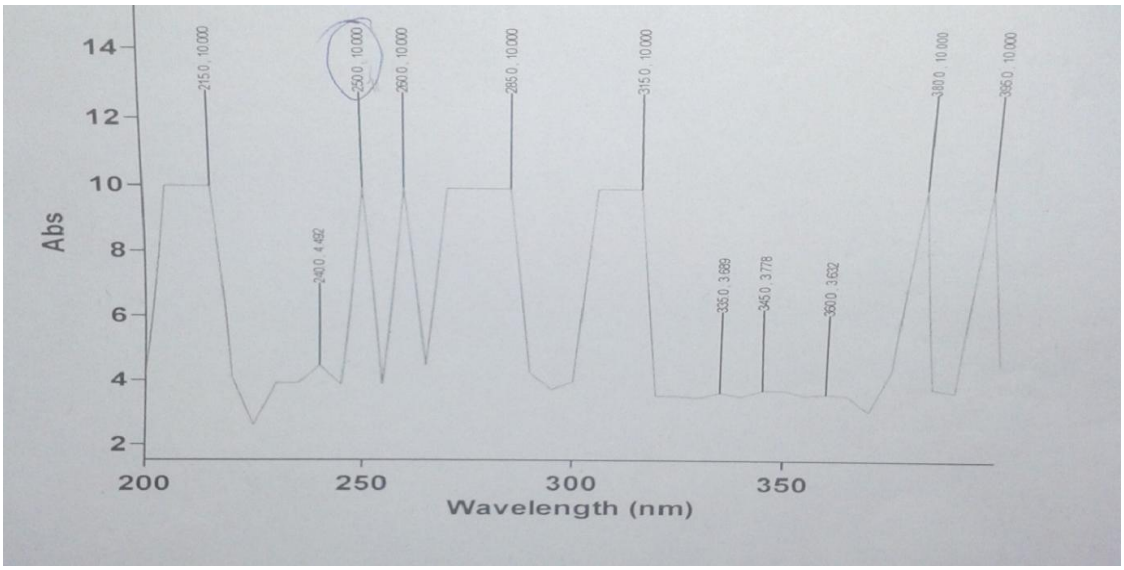

Figure 1: UV examination of Kumari Asava

Table 14: UV examination for absorbance of sample drug

S. No.	Standard range (nm)	Observed range (nm)
1	250-400	250

Table 15: Colorimetric analysis

S. No.	Observed range (nm)
1	240

Table 16: Acid value

S. No.	Acid Value (S.E.M.)
1	9.252 ± 0.03

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Table 17: Determination of pH

S. No.	pH (in 1%)
1	4.7

- Establishing the safety for heavy metal test

Table 18: For cadmium

Experiment	Observation	Result
NH_4OH add in a sample solution	White ppt is absent	Absence of cadmium
Potassium Ferro cyanide is added	White ppt is absent	Absence of cadmium

Table 19: For bismuth

Experiment	Observation	Result
NH_4OH add in a sample solution	White ppt is absent	Absence of bismuth
H_2S gas is added	Dark brown ppt is absent	Absence of bismuth

Table 20: For lead

Experiment	Observation	Result
Dil. HCL add in a sample solution	White ppt of $CaCl_2$ is absent	Absence of lead
KI is added	Yellow ppt is absent	Absence of lead

Conclusion

Standardization of Ayurvedic formulations is an important step for the establishment of a consistent biological activity, a consistent chemical profile, or simply a quality assurance program for production and manufacturing of herbal drugs [14].

Present investigation carried out to develop few parameters for quality and purity of Asavas based upon traditional knowledge. Although these studies are preliminary but presented evaluation parameter will be useful for the standardization of Asavas. The Asava were standardize and evaluated by various standardization parameters such as physicochemical standards like total solid, reducing sugar, non reducing sugar, surface tension, specific gravity, solubility, viscosity, boiling point, UV examination for the absorbance of sample drug, colorimetric analysis, acid value, pH determination and establishing the safety for heavy metal test.

It can be concluded that the formulation of Kumari Asava contains all good characters of an ideal Asava and it was found to be harmless, more effective and economic. The marketed samples have been standardize and evaluated on the basis of the above mentioned parameters which show satisfactory results, but the efficacy of the products can only be judged by doing the pharmacological & clinical methods, which is suggested as future scope of R&D.

The study shows that the contents of formulation presents within the permissible limits as per WHO, could helpful in authentification are not specified in the standard literature such as in pharmacopoeia, which could helpful in authentification of Kumari Asava. The result of present study will also serve as reference monograph in the preparation of Asavas.

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