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Quality Assessment of "*Kanakasava*", an Anti-Asthmatic Ayurvedic Formulation

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ABSTRACT

Asthma is one of the most common chronic diseases affecting an estimated 300 million people worldwide and ranks third responsible for hospitalization. In developing regions (Africa, Central and South America, Asia), asthma prevalence is rising sharply with increasing urbanization and westernization. Plant-based medicines are the 3rd most popular choice of both adults (11 %) and children (6 %) suffering from asthma. WHO encourages, recommends and promotes traditional herbal medicines and their formulations in National Health Care Programme which need to ensure quality control using modern techniques applying suitable standards. Asavas are polyherbal Ayurvedic formulations mentioned in Bhaishajya Ratnawali and its modern English translated version known by the name Ayurvedic Pharmacopoeia of India. *Kanakasava*, a polyherbal Ayurvedic asava formulation, consists of *Datura metel* Linn. *Adhatoda vasica* Nees., *Glycyrrhiza glabra* Linn., *Piper longum* Linn., *Solanum xanthocarpum* Scrad & Wendl, *Zingiber officinalis* Rosc., *Clerodendrum serratum* (Linn.) Moon, *Mesua ferrea* Linn, *Abies webbiana* Lindl., *Woodfordia fruticosa* Kurz., has been used traditionally since ages for the treatment of asthma. Standardization of the formulation was done as per API. The results of standardization parameters of pH (3.85 ± 0.029), specific gravity (1.046 ± 0.009), viscosity (1.52 CS ± 0.006), phenolic content (0.079 % *w/v* ± 0.012%), alcohol content (7.18% *v/v* ± 0.577), total solid content (14.64 % *w/v* ± 0.348) and estimation of heavy metals, aflatoxin, pesticide residues, microbial load complies with the official limits.

Keywords: Asthma, traditional, *Kanakasava*, polyherbal, Ayurvedic, Standardization.

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INTRODUCTION

Asavas are self-generated hydroalcoholic fermented infusions[1], being traditionally used in Ayurveda. Though asavas are regarded as valuable therapeutics due to their efficacy and desirable features, yet least exploited because of their monotonous standardization. They are moderately alcoholic (up to 12% by volume) and sweetish with slight acidity and agreeable aroma. Presence of alcohol in the preparation shows several advantages, like better keeping quality, enhanced therapeutic properties, improvement in the efficiency of extraction of drug molecules from the herbs and improvement in drug delivery into the human body sites[2] of various asava formulations mentioned in Bhaishajya Ratnawali, Kanakasava is an anti-asthmatic ayurvedic polyherbal formulation containing Datura as one of the main ingredient[3]. Here, an attempt has been made to establish a standardized routine procedure for the preparation and standardization of Kanakasava. Prepared formulation was standardized for preliminary and physicochemical parameters like pH, viscosity, solid content, alcohol content, and total phenolic content. Based on the study, the formulation has been characterized and a few salient features of the Kanakasava has been recorded which would facilitate the identification of formulation. Preliminary and physical standards give valuable information for further investigations.

MATERIALS AND METHODS

Collection of raw materials

All the crude drugs and others ingredients (Sugar and Honey) required for the preparation of proposed formulation were collected from the appropriate sources and authenticated by Dr. H. B. Singh, NISCAIR, Delhi, Ref. No.: NISCAIR/RHMD/Consult/-2013-12/1752/52, 2013-12/1821/121. Voucher specimens were deposited in RHMD, NISCAIR for further reference.

All the ingredients were of pharmacopoeial quality and quantity Table 1.

Table 1: Formulation compostion of Kanakasava (Bhaishajya Ratnawali)

S.No	Name of Plant	Part of plant	Place of collection	Time of collection
1.	*Datura metel* Linn.	Whole plant	Jamia Hamdard campus	June
2.	*Adhatoda vasica* Nees.	Root	Jamia Hamdard, Botanical garden	June
3.	*Glycyrrhiza glabra* Linn.	Roots	Jamia Hamdard, Botanical garden	June
4.	*Piper longum* Linn.	Fruits	Local market	June
5.	*Solanum xanthocarpum* Scrad & Wendl.	Whole plant	Jamia Hamdard, Botanical garden	June
6.	*Mesua ferrea* Linn.	Stamens	Pune	June

7.	*Zingiber officinalis* Rosc.	Rhizomes	Local market	June
8.	*Clerodendrum serratum* (Linn.) Moon.	Roots	Tiruvendrum	July
9.	*Abies webbiana* Lindl.	Leaves	Kashmir hills	July
10.	*Woodfordia fruticosa* Kurz.	Flowers	Jamia Hamdard, Botanical garden	July
11.	*Vitis vinifera* Linn.	Dried fruit	Local market	June
12.	Sugar	-	Local market	
13.	Honey	-	Local market	
14.	Water	Distilled	Distillation instrument	

Preparation of the proposed formulation

The proposed formulation was prepared as per the traditional fermentation method as described in Ayurvedic Pharmacopoeia of India (API), Part II[3]. All ingredients were washed, dried in shade, powdered and sieved through sieve # 44. Coarsely powdered drugs were added to formulation vessel, a porcelain jar fumigated with pippali churna & smeared with ghee, containing sugar solution prepared with quantity mentioned in the formula and filtered through muslin cloth. At the end honey, draksha and dhatki pushpa were added and container was sealed with a clay smeared cloth wounded in seven consecutive layers. Container was shifted to an isolated chamber filled with paddy specially prepared for fermentation. The chamber was maintained at 25 ± 2°C and 45 ± 5 % relative humidity. The process of completion of formulation was checked on 8th, 15th, 22nd, 30th and some 35th day for the parameters as signal for completion of the fermentation process. Prepared formulation was filtered through a clean muslin cloth, packed in amber colored air tight glass container and standardized for some important quality standard parameters Table 2.

Table 2: Check parameters for the completion of the fermentation for formulation

Test parameter	**Results**				
	Day8	**Day 15**	**Day 22**	**Day 30**	**Day 34**
Release of CO_2	Burning	Burning	Burning	Burning	Extinguished
pH	6.05	5.56	4.79	4.01	3.85 ± 0.029

Quality standards parameters of formulation

Preliminary evaluation:

The prepared Kanakasava was evaluated organoleptically for its odour, taste, colour and clarity.

Physico-chemical evaluation:

Determination of pH:

pH was determined by the digital pH meter calibrated at pH[4].

Determination of specific gravity:

Specific gravity of formulation was determined using Pycknometer[4].

Determination of viscosity:

Viscosity of the prepared formulation was determined using a U tube viscometer[5]. The kinematic viscosity in centistokes was calculated from the following equation:

Kinematic viscosity = kt

Here, k = viscometer constant determined from liquids of known viscosity, water and glycerine; and t = time in seconds for meniscus to pass through the two specified marks.

Determination of alcohol content:

Distillation method was followed for estimation of alcohol contents[6]. Briefly, 25 ml of the prepared formulation was mixed with 100 ml water, then saturated with sodium chloride, extracted with Hexane, aqueous saline extract collected, made alkaline with NaOH and distilled to 90 ml. The volume was made upto 100ml. On the basis of specific gravity, the yield was calculated on percentage Weight / ml. basis by formula:

% Alcohol content = specific gravity x 4

Determination of total solid content:

Accurately measured 50 ml of the prepared formulation was evaporated at 105 °C extracted with dehydrated alcohol dried and mixed with 1 gm diatomite and again dried at 105°C to constant weight. Total solid content was calculated from the formula[7]:

Weight of the total solids = (weight obtained – 1 gm diatomite) x 100/50

Determination of total phenolic content:

Total phenolic content was determined by method Pourmorad *et al.*, 2006[8], as gallic acid equivalent from the regression equation obtained, Table 3, fig. 1.

Determination of toxic contaminants

Microbial Load Determination[9]:

Total fungal count:

Total fungal count was determined by incubating the mixer of formulation phosphate buffer and liquefied potato dextrose agar medium at 25°C for 7 days. Numbers of colonies were observed and counted.

Total bacterial count:

Determination of Total bacterial count was done by incubating 1ml of the mixer obtained by suspending 1 ml of formulation sample with 100 ml of buffered sodium chloride-peptone solution pH 7, 0.1% w/v of polysorbate 80 in liquefied casein soyabean digest at 30°C to 35°C for 4 days. Numbers of colonies were observed and counted.

Toxic metal determination[10]:

Quantity of heavy metals like arsenic, lead, cadmium and mercury were determined as per method in Ph. Eur. chapter 2.2.58(8) using Perkin Elmer Elan 6000 ICP-OES equipped with an As-91 auto sampler. Instrument was calibrated using reference standards of 1ppm and 10ppm.

Pesticides residues[11]:

Pesticide residues were carried out by standard methods AOAC 970.33 Using Thermo Finnigan GCMSMS equipped with DB-5 fused silica capillary column (30m X 0.25 mm i. d., 0.25 µm film thicknesses). Helium gas at a flow rate of 1.0 ml/min as a carrier gas and Ion Trap detector Type Mass Spectrometer was used. The injection port was maintained at 250° C, and the split ratio was 40:1. Oven temperature programming was done from 60° C hold 1.5 min, 60 to 120 @15°C/min, 120 to 220 @8° C/min, 220 to 280 @5° C/min, Hold 5 min. Interface temperature was kept at 250° C. Ionization source temperature was at 230°C, and 70 eV electron impact modes were employed. Ionization mode was electron Impact ionization and the scanning range was from 40 amu to 400 amu. Pesticide residues standard (organochlorides, organophosphates &pyrethins groups) were procured from sigma (Aldrich).

Aflatoxins analysis[11]:

Agilents LC/MS/MS (Model: 6410B) was used with RRLC Column: C18, 50mm X 2.1mm, 1.8 µm particle size and maintained 40ºC. Mobile Phase was used as 0.1% formic acid + 5 mM ammonium acetate in water and Methanol at a flow rate of 0.2 ml/min. Mass Spectrometer QQQ detector type was used. Aflatoxins standards solutions were obtained from sigma (Aldrich) and kept in at 20°C in a colored amber vial.

RESULTS AND DISCUSSION

Preliminary and Physico-chemical evaluation:

All the results obtained from Preliminary evaluation and Physico-chemical evaluation analyses are summarized in the Table 4 & 5 respectively.

Table 4: Preliminary evaluation:

S.No.	Parameters	Method	Results
1.	Color	Natural light	Brown
2.	Odour	Sensory	Alcoholic
3.	Taste	Palatability	Sweet and alcoholic

Table 5: Physico-chemical evaluation

S. No.	Parameters	Results
1.	pH	3.85 ± 0.029
2.	Specific gravity	1.046 ± 0.009
3.	Viscosity	1.52 CS ± 0.006
4.	Alcohol content	7.18% *v/v*± 0.577

5.	Total solid content	14.64 % *w/v* ± 0.348
6.	Total phenolic content	0.079 % *w/v* ± 0.012%

Total phenolic content:

Total phenolic content determined as gallic acid equivalent was 0.0787 ± 0.012%. Results of absorbances of standard gallic acid at 765 nm are shown in table 3 and figure 1.

Table 3: Absorbance of gallic acid standard at 765 nm

Conc. of standard solution of gallic acid (µg/ml)	Absorbance recorded at 765 nm
10	0.155
20	0.252
30	0.403
40	0.521
50	0.691
100	1.119
150	1.751
Sample 1	0.072
Sample 2	0.081
Sample 3	0.079
Mean conc.	**0.0787±0.012%**

Calibration curve of gallic acid standard

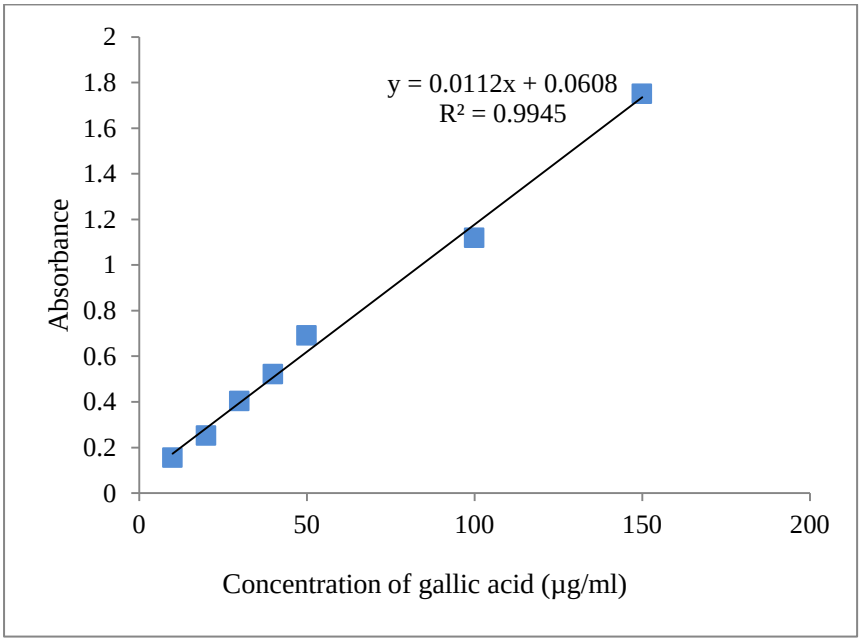

Figure.1. Calibration curve of gallic acid standard

Microbial Load Determination:

The total fungal count and total bacterial count was found to be less than 1 colony forming units (cfu) that shows that formulation has not any microbial growth as shown in table 6.

Table 6: Microbial Load Determination

S. No.	Test parameter	Results
1	Total Bacterial Count, cfu/ml	<1.0
2	Total fungal Count, cfu/ml	<1.0

Toxic contaminant determination:

The toxic contaminants viz., heavy metals (cadmium, mercury, lead and arsenic) and Aflatoxins found to be less than the European Pharmacopoeial limit as mentioned in tables 7 & 8 respectively. Pesticides residues were found to be absent in the formulation. The results reveal that all the toxic contaminants are within the pharmacopoeial permissible ranges.

Table 7: Heavy metal analysis

S. No	Test parameter	Results	Ph. Eur. Draft monograph Herbal drugs(2008) [11]
1	Cadmium	Not detected	0.5 mg/kg
2	Lead	0.3 mg/kg	5 mg/kg
3	Mercury	Not detected	-
4	Arsenic	Not detected	0.1 mg/kg

Table 8: Results of Aflatoxin determination

S. No.	Test parameter	Results	MDL*
1	Aflatoxin B1	Below Detection Limit	1.0µg/kg
2	Aflatoxin B2	Below Detection Limit	1.0µg/kg
3	Aflatoxin G1	Below Detection Limit	1.0µg/kg
4	Aflatoxin G2	Below Detection Limit	1.0µg/kg

*****Maximum Detection Limit

CONCLUSION

The formulation under study was subjected to physicochemical analyses which are helpful in establishing the standard parameters. Established preliminary and physical standards give valuable information for further investigations and facilitate the identification of formulation in routine industrial production.

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