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Research Article

Quality Standard Parameters of an Anti-Asthmatic Ayurvedic Formulation "*Kanakasava*"

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ABSTRACT

The aim of the present study is to optimize the method of preparation of herbal anti-asthmatic Ayurvedic formulation "Kanakasava" and to standardize the prepared formulation by important pharmaceutical parameters. *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. Since ages the formulation has been used traditionally for the treatment of asthma. Method of preparation was optimized for pH and CO_2 release as check parameters for completion of fermentation and prepared formulation was standardized for preliminary physico-chemical parameters as per Ayurvedic Pharmacopoeia of India. The formulation was also investigated for toxicological determinants like pesticides residues, heavy metals, aflatoxins and microbial growth. The results of standardization i.e., pH (3.86 ± 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 toxicological determinants complies with the official limits. Results show that the prepared formulation is characterized for physico-chemical parameters that would facilitate the identification of formulation for further research.

Key words: Asthma, *Kanakasava*, polyherbal, Ayurvedic, Standardization

INTRODUCTION

Asthma is one of the most common chronic diseases affecting an estimated 300 million people worldwide[1] and ranks third responsible for hospitalization.[2]. 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 self-generated hydroalcoholic fermented infusions, 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.[3] Of various asava formulations mentioned in Bhaishajya Ratnawali, Kanakasava is an anti-asthmatic ayurvedic polyherbal formulation containing Datura as one of the main ingredient. 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.

Preparation of the proposed formulation: Traditional method as described in Ayurvedic Pharmacopoeia of India (API), Part II was followed for preparation of the proposed formulation.[4] Briefly all the crude drugs were washed, shade dried, powdered and sieved through sieve # 44. Coarsely powdered drugs were added to sugar solution contained in a porcelain jar. At the end honey, draksha and

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Table 1: Formulation composition 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	

Table 2: Parameters for the completion of fermentation

Test parameter	Day 8			Day 15			Day 22			Day 30			Day 34		
	B_1	B_2	B_3	B_1	B_2	B_3	B_1	B_2	B_3	B_1	B_2	B_3	B_1	B_2	B_3
Release of CO_2	B	B	B	B	B	B	B	B	B	B	B	B	Ex	Ex	Ex
pH	6.07	6.05	6.02	5.55	5.56	5.58	4.72	4.79	4.84	4.08	4.01	3.94	3.85	3.45	3.55

**Note: B_1: batch 1; B_2: batch 2; B_3: batch 3; B: burning.*

Table 3: Preliminary evaluation

S.	Parameters	Method	Results
1.	Color	Natural light	Dark brown
2.	Odour	Sensory	Alcoholic
3.	Taste	Palatability	Sweet

dhatki pushpa were added and container was sealed with a clay smeared cloth. Container was kept at an isolated chamber maintained at 25 ± 2°C and 45 ± 5 % relative humidity. The process of completion of formulation was checked on 8th, 15th, 22nd, 30th and some35th day for some important parameters that act as signals for completion of the fermentation process.[5] 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 3 and 4. Three batches of formulation were prepared and each batch was checked for following parameters to assure the process of completion of fermentation Table 2:

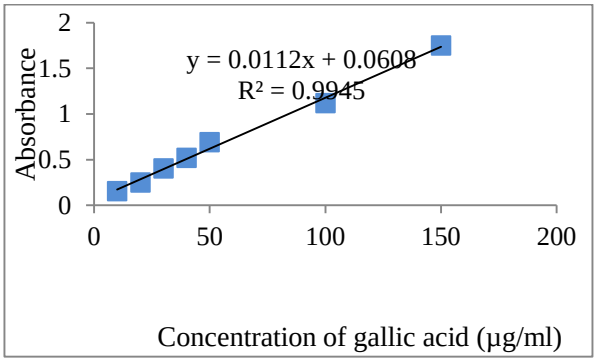

Fig.1. Calibration curve of gallic acid standard

Standardization parameters of formulation

Preliminary evaluation: Different batches of prepared formulations were evaluated organoleptically for their colour, odour, taste and clarity.

Physico-chemical evaluation

Table 4: Physico-chemical evaluation

S. No.	Parameters	Results
1.	pH	3.86 ± 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%

*Note: *All readings are % Mean ± S.D, n=3*

Determination of pH: pH for different batches were determined by a digital pH meter calibrated at pH= 6. [6]

Determination of specific gravity: Specific gravity of different batches of formulations was determined using Pycnometer[6].

Determination of viscosity**:** Viscosity of all the three batches of prepared formulations was analysed using a U tube viscometer.[7] 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

Table 5: 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%

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

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

seconds for meniscus to pass through the two specified marks.

Determination of alcohol content: Alcohol content was determined using general distillation method with some modifications. Briefly, 25 ml of the prepared formulation was mixed with 100 ml water. Mixture was saturated with sodium chloride and extracted with hexane. Aqueous saline extract was collected, made alkaline with NaOH and distilled to 90 ml. Final volume was made up to 100ml.[8] 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: 50 ml of the prepared formulation was evaporated at 105 °C. The residue was extracted with dehydrated alcohol, dried and mixed with 1 gm diatomite and dried at 105 °C to constant weight. Total solid content was calculated from the formula.[9]

Weight of the total solids =

(weight obtained – 1 gm diatomite) x 100/50

Estimation of total phenolic content: Total phenolic content was determined by the method of Pourmorad *et al.,* 2006.[10] The phenolic content was measured as gallic acid equivalent from the regression equation of standard gallic acid, Table 5, fig. 1.

Determination of toxic contaminants

Microbial Load Determination[11]

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. Developed colonies were observed and counted.

Total bacterial count: For determination of total bacterial count 1ml of formulation was suspended 1 with 100 ml of buffered sodium chloride-peptone solution and incubated at pH 7, with 0.1% w/v of polysorbate 80 in liquefied casein soyabean digest at 30°C to 35°C for 4 days. Number of developed colonies were observed and counted.

Toxic metal determination[12]: 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[13]: Pesticide residues were carried out by standard methods AOAC 990.33 Using Thermo Finnigan GCMS/MS equipped with DB-5 fused silica capillary column (30m X 0.25 mm i. d., 0.25 µm film thicknesses). Carrier gas and detector type was helium at a flow rate of 1.0 ml/min and Ion Trap Mass Spectrometer. The injection port was maintained at 250°C and the split ratio was 40:1. Oven temperature programming was done at 60° C for 1.5 min, 60 to 120 @15°C/min, 120 to 220 @8° C/min, 220 to 280 @5° C/min, holding time of 5 min. Interface temperature was maintained at 250° C but Ionization source temperature was at 230°C and electron impact mode was employed at 70 eV. Scanning range was between 40 amu to 400 amu. Pesticide residues standards (organochlorides, organophosphates and pyrethins groups) were procured from sigma (Aldrich).

Aflatoxins analysis[13]: Agilents LCMS/MS (Model: 6410B) was used with RRLC Column: C18, 50mm x 2.1mm, 1.8µm particle size. 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 at -20° C in a colored amber vial.

RESULTS AND DISCUSSION

Preliminary and Physico-chemical evaluation: All the results obtained from Preliminary physico-chemical analyses are summarized in the Table 3 &4 respectively.

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 5 and fig 1.

Table 8: Results of Aflatoxin determination

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

***** *BDL: Below detection limit; MDL: Maximum Detection Limit*

Table 9: Pesticides residues analysis

S.No.	Pesticides	Test Method	Result	MDL
1.	α-BHC	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
2.	β-BHC	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
3.	γ-BHC(Lindane)	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
4.	δ-BHC	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
5.	Heptachlor	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
6.	Heptachlor_Epoxide	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
7.	α-Chlordane	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
8.	α-Endoulfan	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
9.	β-Chlordance	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
10.	Endrin	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
11.	Total DDE	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
12.	Total DDD	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
13.	Total DDT	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
14.	β-Endoulfan	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
15.	Endrin _Aldehyde	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
16.	Endoulfan_sulfate	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
17.	Aldrin	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
18.	Endrin_Ketone	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
19.	Methoxychlor	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
20.	Dieldrin	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
21.	Alachlor	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
22.	Butachlor	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
23.	Monocrotophos	AOAC 970.52/EPA 525.2	Not Detected	0.05 mg/kg
24.	Phorate	AOAC 970.52/EPA 525.2	Not Detected	0.05 mg/kg
25.	Mevinphos	AOAC 970.52/EPA 525.2	Not Detected	0.05 mg/kg
26.	Dimethoate	AOAC 970.52/EPA 525.2	Not Detected	0.05 mg/kg
27.	Malathion	AOAC 970.52/EPA 525.2	Not Detected	0.05 mg/kg
28.	Methyl-parathion	AOAC 970.52/EPA 525.2	Not Detected	0.05 mg/kg
29.	Chlorpyrifos	AOAC 970.52/EPA 525.2	Not Detected	0.05 mg/kg
30.	Ethion	AOAC 970.52/EPA 525.2	Not Detected	0.05 mg/kg
31.	Atrazine	AOAC 970.52/EPA 525.2	Not Detected	0.05 mg/kg
32.	Simazine	AOAC 970.52/EPA 525.2	Not Detected	0.05 mg/kg
33.	Diazinon	AOAC 970.52/EPA 525.2	Not Detected	0.05 mg/kg
34.	Phosphamidon	AOAC 970.52/EPA 525.2	Not Detected	0.05 mg/kg
35.	Fenitrothion	AOAC 970.52/EPA 525.2	Not Detected	0.05 mg/kg
36.	Fenthion	AOAC 970.52/EPA 525.2	Not Detected	0.05 mg/kg
37.	Phosalone	AOAC 970.52/EPA 525.2	Not Detected	0.05 mg/kg
38.	Quinalphos	AOAC 970.52/EPA 525.2	Not Detected	0.05 mg/kg
39.	Coumaphos	AOAC 970.52/EPA 525.2	Not Detected	0.05 mg/kg
40.	Parathion	AOAC 970.52/EPA 525.2	Not Detected	0.05 mg/kg
41.	Malaoxon	AOAC 970.52/EPA 525.2	Not Detected	0.05 mg/kg
42.	Dichlorvos	AOAC 970.52/EPA 525.2	Not Detected	0.05 mg/kg
43.	2,4-D	PAM Vol I / EPA 515.3	Not Detected	3 mg/kg
44.	Hexachlorobenzene	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg

** Maximum Detection Limit*

Microbial Load Determination: The results of total fungal count and total bacterial count are mentioned in table 6. The results show that formulation is devoid of any microbial growth.

Toxic contaminant determination: The results of heavy metals (arsenic, cadmium, lead and mercury) , aflatoxins and pesticides residues analyses are mentioned in tables 7, 8 and 9 respectively. The results show the presence of heavy metal less than the European Pharmacopoeial limit and absence of any pesticides residues in the formulation. The results reveal that all the toxic contaminants are within the pharmacopoeial range.

CONCLUSION

Established preliminary and physicochemical standards give important information for further investigations and

facilitate the identification of formulations in routine industrial production.

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