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Quality evaluation and standardization of *Adraka Khanda:* An Ayurvedic formulation

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INTRODUCTION

Ayurveda, the antique therapeutic system of India promotes a combination of lifestyle management (which includes diet, exercise and medication) and treatment with specific herbs and minerals to cure various diseases. The effect of Ayurvedic drugs are purely based on surveillance and seem subjective without valid scientific backing. Global resurgence of Ayurveda needs its scientific validation both in terms of efficacy and safety [1]. Standardized drug manufacturing is the primary and basic step in this regard. Reliable quality control protocols for Ayurvedic formulations using modern techniques of analysis are extremely important [2]. Present scenario has changed now as compared to the ancient time. Presently the medicines of Ayurvedic system of medicine are being manufactured on the huge level in Pharmaceutical companies, where manufacturers come across many problems such as qualitative and authentic raw drugs, Good Manufacturing Practices (GMP), availability of standards as well as proper protocol for standardization of both medicinal plants and developed formulations [3]. There are many therapeutic forms of drugs described in classical texts, like *Asava, Arishta, Ghruta, Taila, Churna, Vati, Gutika, Kwatha* and much more [4]. In ancient time Ayurvedic medicines were prepared by physician itself according to the roga-rogi prakriti. Now a day it is manufactured in pharmacies. Therefore, it is very important to standardize the formulation not only on modern ground but also on ancient ayurvedic aspect methodologies of formulation [3]. *Sneha siddha* [*Ghrita* (clarified butter) or oil based preparation] have better pharmacokinetic properties (absorption, distribution, metabolism and excretion) in comparison to other dosage forms because of the lipophilicity of the cell membrane, as it readily permeate lipid soluble substances into the cells [6]. Standardization is a necessary issue for polyherbal preparations in order to evaluate the quality of the drugs based on the quantification of their active principles. India, as place of origin of the traditional system of health care may play a leading role in the making of standardized, therapeutically effective Ayurvedic formulations. WHO support, advocate and encourage different traditional medical system as a part of national health policies due to easy availability, low cost, safety and faith of people in them [1]. The World Health Assembly in its different resolutions highlighted the requirement of the quality natural products by using modern control techniques and applying suitable standards [7].

Bhaishajya Ratnavali, a well known book for Ayurvedic formulations mentioned *Adraka Khanda* in the management of skin diseases like *Shitpitta* (Urticaria) [8]. The present paper reports the preparation and standardization of *Adraka Khanda* based on organoleptic characters, physical characteristics, and physicochemical properties. Different analytical techniques were employed in quality control and standardization of formulation.

EXPERIMENTAL SECTION

Plant material

Crude drugs were purchased from the local market of Varanasi (Uttar Pradesh) (25°20' N, 83°00' E., 80.71 mtrs. ASL) and authenticated by Prof. A. K. Singh, Professor and Head, Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University Varanasi.

Method of preparation of *Adraka Khanda*

Adraka Khanda was prepared as per the procedure mentioned in *Bhaishjyaratnavali* [8]. All the herbal ingredients of Pharmacopoeial quality present in the formulation were mentioned in Table 1.

Table 1. Ingredients of Adraka Khanda

S. N.	Name	Botanical Name	Family	Part Used	Quantity
1	*Adraka*	*Zingiber officinale* Rosc.	Zingiberaceae	Rhizome	1 kg
2	*Chitraka*	*Plumbago zeylanica* Linn.	Plumbaginaceae	Root Bark	50 g
3	*Ela*	*Elettaria cardamomum* Maton.	Zingiberaceae	Fruit	50 g
4	*Karchura*	*Curcuma zedoaria* Rosc.	Zingiberaceae	Rhizome	50 g
5	*Maricha*	*Piper nigrum* Linn.	Piperaceae	Fruit	50 g
6	*Mustaka*	*Cyperus rotundus* Linn.	Cyperaceae	Rhizome	50 g
7	*Nagakeshara*	*Mesua ferrea* Linn.	Clusiaceae	Fruit	50 g
8	*Patra*	*Cinnamomum tamala* Nees & Eberm.	Lauraceae	Leaves	50 g
9	*Pippali*	*Piper longum* Linn.	Piperaceae	Fruit	50 g
1	*Pippali Mula*	*Piper longum* Linn.	Piperaceae	Fruit	50 g
1	*Shunthi*	*Zingiber officinale* Rosc.	Zingiberaceae	Rhizome	50 g
1	*Twaka*	*Cinnamomum zeylanicum* Blume.	Lauraceae	Bark	50 g
1	*Vidanga*	*Embelia ribes* Burm. f.	Myrsinaceae	Fruit	50 g
1	*Goghrita*	-	-	-	400 g
1	*Godugdha*	-	-	-	2 L
1	*Sharkara*	-	-	-	1 kg

All the raw plant materials except fresh ginger were cleaned, dried in an oven at 45ºC, tested for foreign matter and stored in the air tight containers. Further the drugs were powdered separately and sieved through sieve 85#. Then these powders were mixed thoroughly in a specified quantity to obtain a homogeneous mixture (*Prakshepa dravya*). Fresh ginger was cleaned by washing with lukewarm (40–50°C) water, peeled, and small pieces of ginger were ground into a paste using mechanical grinder. Then ginger paste was fried in Goghrita maintaining the temperature between 80 to 90°C till ginger turned brown and its typical smell emanate; after that Godugdha was added and stirred. Sugar was dissolved in the water and then the sugar solution was strained to remove the foreign particles. Sugar solution was mixed to fried ginger and heated together on a mild flame with continuous stirring till the desired thickness and consistency was obtained. After that mixture of fine powder (*Prakshepa dravya*) was added and mixed thoroughly to prepare a homogeneous blend. Then the blend was passed through 40# sieve to obtain granules which was then dried at room temperature. The granules were then packed in airtight containers.

Physico-chemical evaluation

The organoleptic characters and powder microscopy were studied as per standard procedure. Microphotographs were taken using Magnus Microscope Image Projection System (MIPS). The colour changes of the drug powder with respect to different chemical reagents were observed under short UV (λ_{max} 254 nm) and long UV (λ_{max} 366 nm) as per the standard procedures [9-11]. The identification and comparison of the colors was done using the standard colour index chart. Physico-chemical characters such as percentage of ash values, extractive values, pH value and pesticide contamination of *Adraka Khanda* were performed according to the official methods [12-13].

Preliminary phytochemical evaluation

The 5 g of formulation was extracted individually with methanol, hexane, chloroform, ethyl acetate and water (100 mL each) using cold maceration process for 24 h (frequent shaking for 6 h and then permitted to stand for 18 h). The extracts were filtered and concentrated in rotary evaporator (Perfit India, Pvt. Ltd.) below 60°C to generate the extracts of *Adraka Khanda* and were finally stored in dessicator for further studies. Preliminary phytochemical screening for the presence of various phytoconstituents such as alkaloids, carbohydrate, steroids, glycosides, saponins, terpenoids, phenolics, flavonoids and protein were carried out by using standard procedures [14-15]. Presence of phytochemicals was further confirmed using thin layer chromatography (TLC). Silica gel 60 F_{254} was used as stationary phase [16] and mixture of different solvents as Mobile phases.

Determination of Saponification value

Dissolve 40 g of potassium hydroxide in 20 mL water and alcohol was added to make 1,000 mL. It was allowed it to stand overnight and the clear supernatent was poured off. About 2 g of the substance was then weighted in a tared 250 mL flask and 25 mL of the alcoholic solution of potassium hydroxide was added. It was then attached to a reflux condenser and boiled on a water-bath for one hour, frequently rotating the contents of the flask. The mixture of then cooled and 1 mL of solution of phenolphthalein was added after that the excess of alkali has been titrated with 0.5 N hydrochloric acid. Note the number of mL required **(a)** Repeat the experiment with the same quantities of the same reagents in the manner omitting the substance [17]. Note the number of mL required **(b)** Calculate the saponification value from the following formula

Saponification Value = (b–a) × 0.02805 × 1000/ W

Where 'W' is the weight in g of the substance taken.

Quantification of phytoconstituents

Various phytochemicals present in the *Adraka Khanda* was quantified for total phenolic [18], total tannin [19], flavonoid and flavonol content [20].

Total phenolic compound analysis

Quantification of total polyphenol was done by Folin-Ciocalteu (FC) assay method with Gallic acid as a reference standard. 1.0 mL of extract solution (10 mg/mL) was mixed with 0.8 mL of 2% Na_2CO_3 and 1.0 mL of FC reagent (diluted 1:10 with de-ionized water). Further water- methanol (4:6) was used as diluting agent to make the volume up to 10 mL. The solution was allowed to stand for 30 min then absorbance was measured at 765 nm using spectrophotometer. The total phenolic content was expressed in mg/g gallic acid equivalent (GAE) of dry extract [18].

Total tannin estimation

0.5 mL of extract solution (10 mg/mL) was mixed with 0.8 mL of 2% Na_2CO_3 and 5.0 mL of Folin- Denis reagent (FD). The volume was made up to 100 mL. The mixture was mixed well and kept on room temperature for 30 min then measured the absorbance at 760 nm. Total tannin content as expressed as mg tannic acid equivalent /100 g of sample [19].

Determination of total flavonoids

Dowd method was adapted for quantitative estimation of the total flavonoid content. 5.0 mL of extract solution (10 mg/mL) was mixed with 5.0 ml of 2% aluminium trichloride ($AlCl_3$) in methanol and kept for 10 min. Absorbance was taken at 415nm. A blank solution contains extract and methanol was used for comparison. Total flavonoid content is expressed as g of rutin equivalents / 100 g of sample [20].

Microbial Contamination

Microbial contamination and total viable aerobic count were determined in 1 month old sample using Mac. Conkey and soyabean-casein digest mediums as per method described by WHO [13].

Heavy Metal Analysis and Pesticide Residue Evaluation

Wet digestion method was adopted for sample preparation. 2 g of the sample was treated with 10 mL of HNO_3 v/v in a 100 mL beaker. It was then kept on a hot plate at 95°C until it was free from brown fumes of nitric acid. 5 ml of concentrated nitric acid was added after cooling and further heated for 30 min at 95°C. This last step was repeated and reduced the volume of solution to about 5 ml without boiling. It was cooled and added with 2 ml of deionized water and 3 mL of hydrogen peroxide (30 % v/v). When reaction was completed, the solution was added with 5 mL of conc. HCl, 10 mL of deionized water and heated for 15 min. The solution was cooled and finely diluted up to 50 ml with water. The digested solutions were analyzed through AAS (Atomic absorption spectroscopy) (Shimadzu–AA6300). Each sample was tested thrice. The limits of quantification will be lead (10ppm), arsenic (3ppm), mercury (3ppm) and cadmium (3ppm) [21]. Pesticide residue was evaluated as per WHO guideline [13, 22].

RESULTS AND DISCUSSION

AK is an important ghee based pharmaceutical preparation mentioned in Ayurvedic literature. Such type of formulations involves complex mechanism, where *Ghrita*, which is basically triacylglycerols interacts with other phytoconstituents of paste or decoction drugs and undergoes hydrolysis resulting in the formation of fatty acid and glycerol [23]. These fatty acids are Amphipathic in nature (hydrophobic exterior and hydrophilic interior) interacts with both type of constituent (polar and nonpolar). The continuous heating and agitation during the preparation of AK enhances the extraction process by weakening the hydrogen bonds thereby separating the hydrophobic component from hydrophilic component [24]. Moreover, amphipathic lipids present in an aqueous media facilitated micelles formation. The hydrophobic matter acts like surface active agents which are soluble in fatty material after the evaporation of water. Hence with micelle formation, the finished product is found likely to contain oil soluble as well as water soluble active principles. Digestion, absorption and delivery to a target organ system are facilitated by ghee and helps in achieving the maximum advantage from the formulation [25].

Inhouse formulation *Adraka khand* was prepared by method described in Bhaishjya ratnavali and evaluated as per WHO guidelines. Organoleptic parameters revealed that the formulation was brown in color, with pleasant odor, sweet taste and have a good elegancy as well as appearance (table 2).

Table 2. Macroscopic characteristics

S.N.	Characteristics	Observation
1	Color	Brown
2	Hardness	Rough
3	Odour	Characteristic
4	Taste	Sweet
5	Appearance	Granular

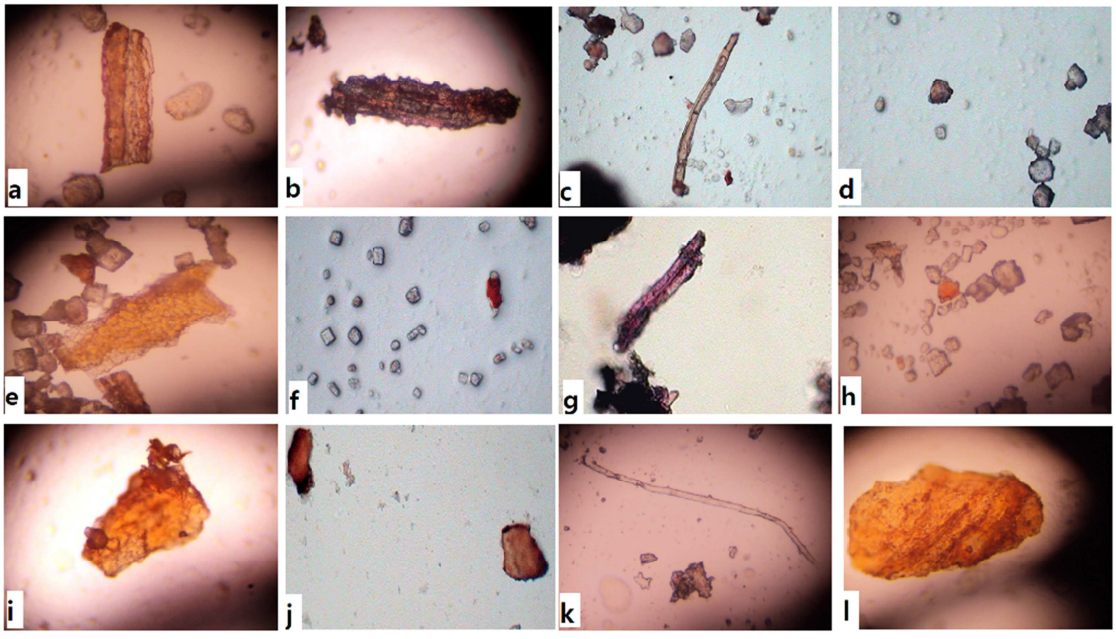

Figure 1. Microscopy of *Adraka Khanda*

[a]: Lignified fibres (Twak); [b]: Lignified fibres (Twak); [c]: Fibers (Chitraka); [d]: Brownish red colouring matter (Vidanga); [e]: Epidermis of seed with overlyning cotyledon cells (Ela); [f]: Brownish red colouring matter (Vidanga) and Acicular calcium oxalate crystals (Pippali); [g]: Lignified fibres (Twak); [h]: Prismatic calcium oxalate crystals (Pippali) and Brownish colouring matter (Nagkeshar); [i]: Obliquely cut clerenchyma of the testa (Ela);[j]: Sclerenchyma of the testa in surface view (Ela); [k]: Fibes (Adraka); [l]: Obliquely cut clerenchyma of the testa (Ela).

Powder Microscopy

Diagnostic microscopic characters of *Adraka Khanda* are Lignified fibres from *Twak;* Fibers from *Chitraka and Adraka*; Brownish red colouring matter from *Vidanga* and Brownish colouring matter from *Nagkeshar*. Acicular as

well as Prismatic calcium oxalate crystals from *Pippali*; Obliquely cut clerenchyma of the testa, Sclerenchyma of the testa along with Epidermis of seed with overlyning cotyledon cells from *Ela* were observed (**Figure 1**).

Physicochemical parameter

The physicochemical parameter of in-house formulation is given in Table 3. The result obtained from phytochemical screening reveals that phytoconstituents like carbohydrates, alkaloid, saponin and flavonoids were present (Table 4). The standardization of *ghrita* formulation indicated that all values are within the standard ranges. The prepared formulation does not show any microbial growth after 1 month. The formulations are free from any toxic material. The results obtained in this study may be considered as tools for assistance to the regulatory authorities, scientific organization and manufacturers for developing standards. Different fluorescence behavior of the AK was enumerated in table 5.

Table 3. Physicochemical evaluation

S.N	Parameter		Results
1.	*Loss on drying (% w/w)*		Not more than 2.375 %
2.	***Ash Values***		
	Total ash (% w/w)		Not more than 1.99 %
	Water soluble ash (% w/w)		Not more than 0.37 %
	Acid insoluble ash (% w/w)		Not more than 1.023 %
3.	***Extractive values***	***Color of extract***	
	Water	Light brown	Not less than 8.31 % (*w/w*)
	Methanol	Dark brown	Not less than 7.70 % (*w/w*)
	Chloroform	Yellowish brown	Not less than 6.55% (*w/w*)
	Ethyl Acetate	Dark brown	Not less than 1.97% (*w/w*)
	Hexane	Dark brown	Not less than 10.19% (*w/w*)
4.	*Saponification value*		56.1
5.	*pH value (1% aqueous solution)*		5.66
6.	*pH value (10% aqueous solution)*		5.96
7.	*Quantitative estimation*		
	Reducing Sugar		*15.67*
	Volatile oil		Not less than 0.5 %
	Crude fiber		Not less than 6.43%
8.	Pesticide residue		
	Chlorinated pesticide residue		
	TS1 (First elute)		Not more than 0.0009 mg/kg
	TS 2 (Second elute)		Not more than 0.011 mg/kg
	Phosphated pesticide residue		
	TS1 (First elute)		Not more than 0.013 mg/kg
	TS 2 (Second elute)		Not more than 0.011 mg/kg
	TS 3 (third elute)		Not more than 0.008 mg/kg
9.	Heavy metals		
	Lead (Pb)		Not more than 0.008 ppm
	Cadmium (Cd)		Not more than 0.0001 ppm
	Zinc (Zn)		Not more than 0.092 ppm
	Mercury (Hg)		Not more than 0.106 ppm

Table 4. Phytochemical screening of different extracts

Plant Constituents Test / Reagent	Aqueous extract	Alcohol extract	Ethyl acetate extract	Chloroform extract
Alkoloids				
Dragendroff's reagent	-	-	+	+
Amino acids	-	-	-	-
Carbohydrate				
Molisch's reagent	+	+	+	+
Fehling solution	+	+	+	+
Cardiac glycosides	-	-	-	-
Flavonoids				
Shinoda/Pew test	-	-	-	+
Saponins				
Foam test	-	-	-	+

(+): Present; (-): Absent

Table 5. Fluorescence analysis of *Adraka Khanda*

Treatment	Long U.V. (λ_{max} 365 nm)	Short U.V. (λ_{max} 254 nm)
NaOH + Methanol	Springgreen	NF
NaOH + Water	Limegreen	NF
HNO_3 + Methanol	Darkkhaki	NF
HNO_3 + Water	Cornflower	NF
HCl + H_2O	Cornflower	NF
HCl + Methanol	Seagreen	NF
Iodine solution	Aquamarine	NF

NF: No Fluorescence

Quantitative estimation of phytoconstituents

Total polyphenols were found to be 153.96 ± 1.25 and 176.04 ± 0.72 mg equivalents to gallic acid/g extract in aqueous and methanolic extract respectively. Antioxidant activities of plant extracts were usually linked to their phenolic content. Total flavonoid contents were found to be 2.58 and 2.07 g of rutin equivalents/100 g of sample in aqueous and methanolic extract respectively. It can be due to higher solubility of ginger flavonoids in water than other solvents. Total Tannin contents were found to be 1.54 and 1.07 g of tannic acid equivalents/100 g of sample in aqueous and methanolic extract respectively.

Saponification value

Saponification value represents the length of fatty acid chain with an inverse relation i.e. longer the chain of fatty acid lower is the saponification value, because long chain fatty acid contains lesser number of carboxylic functional groups per unit mass [23]. The Saponification value of AK demonstrates that the formulation contains medium chain fatty acids as main component which are usually thought to be good for health [26].

Evaluation of Microbial Content

Total Aerobic Organisams (CFU/g) was found to be 1.0 x 10^3 but no visible microbes were observed. As the formulation was prepared with milk there is a chance of growth of microbes after some times but *ghrita* base may protect microbial growth. Microbial contamination may reduce different phytoconstituents with time. Therefore, estimation of microbial load is a necessary step in the quality control of the finished products [27].

Heavy metals analysis

The curiosity about safety aspect of Ayurvedic products have been doubted recently and in many western countries. Ayurvedic drugs have been put on trial and banned on the account of high heavy metal contents, which possess a serious problem for Ayurvedic practioner and Ayurvedic industry [28]. Many reports regarding heavy metal toxicity related to Ayurvedic medicines were published from many areas across the world including the Indian subcontinent, North America, the Middle East, Western Europe and Australia [29, 30]. On prolong ingestion, accumulation of these heavy metals take place in body and create severe health problems [31, 32]. Therefore, from safety point of view, it is necessary to examin the maximum permissible Limit of heavy metals in crude drugs and formulations to improve quality standards for these medicines [33].

With increase in demand and commercial propagation of medicinal plants are promoted now a day. Here for the better outcome and to improve the production of useful parts, farmers use organic input such as pesticides. This in turn also added to contamination of plants and formulations with harmful substances. It has become essential that a uniform standard operating procedure should be development regarding every aspect from propagation of the plant to the formulation development so that the required standards could be met and acceptability of the Ayurvedic products could be enhanced across the globe. All the heavy metals are found within the permissible limits which is justifies the safety of AK in context to heavy metal toxicity.

CONCLUSION

Ayurvedic medicine AK has been standardized by intervention of modern scientific quality control measures in the traditional preparation described in classical texts. Preliminary phytochemical studies, heavy metal analysis shows values are within the limits and there are no harmful metals found. It does not contain any harmful microbes such as *Escherichia coli, Salmonella spp., Pseduomonas aeruginosa, Staphylococcus aureus*. *Adraka khanda* can be give internally to the patients for the management of *Sitapitta* (urticaria). The analytical data generated here may be considered as the standard for this formulation and may help in preserving the quality of drug. Looking at the

growing demand for the herbal drugs in the global market it would be a good idea to use this protocol for other drugs too.

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