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AN ANALYTICAL STUDY OF LAUHA RASAYANA

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ABSTRACT

In Ayurveda the analytical techniques always have been mentioned in classical texts to understand the quality of the end product e.g. "Gatarasatva" of Kwatha Dravyas indicate the completion of Paka. Even since the time of *Veda* physic-chemical properties of various drugs (Like Rasa, Guna, Virya, Vipaka, and Prabhava) have been established. Various refrences are found in Sharanghdhar Samhita and Bhava Prakash also. But with the advancement of science various modern techniques and tools are developed to evaluate and validate formulations scientifically. Here, a step has been made to lay down standard for quality of Lauha Rasayana preparation. After the preparation of Lauha Rasayana, prior to its administration, for safety

and efficacy, were assessed on various analytical parameters. The detailed analytical study and results were documented.

KEYWORDS: Gatarasatva, Lauha Rasayana, Sharanghdhar Samhita.

INTRODUCTION

Increasing demand of ayurvedic products as well as the exponential growth of ayurveda pharmaceutical industry in the global market, has at the same time lead to increasing concerns regarding the safety and efficacy of ayurvedic formulations especially the herbominerals. Technological advancements and apprehensions of modern science obliged the patients and physicians to be watchful about the quality assurance, safety and efficacy of the medicine. Gone are the days when quality of medicine was not subjected to critique but based on the sacred trust existed between the patient and the physician. Quality and genuinity are today's watchwords and hence it is the need of the hour to produce finger prints for quality medicines.

Any medicine is always mandatory to be of highest quality, and hence to check the quality of the finished products and to prove the safety of the drugs on the basis of scientific evidence, it becomes necessary to perform analytical studies of the product. It is evident that any living, evolving system of traditional medicine which has served society for several millennia, could not have survived without possessing quality standards of its own. They have their own sophisticated internal quality standards. They include standards for identity, collection procedures, processing technology, finished products, drug design and therapeutic applications.

Quality of a drug depends upon its formulation, processing, and applications. It is essential to fix some standards for manufacture of drugs so that the genuineness of the drug is not compromised. Ayurveda texts have described several methods for quality control of finished product such as.

AIM AND OBJECTIVES

- 1. To analyze with objective to characterize the *bhasma* preparations- *Lauha*, and *Kasisa*.
- 2. To analyze sample of *Lauha Rasayana* as per classical and modern parameters.

MATERIALS AND METHODS

Preparation of sample

Lauha Rasayana^[1] was prepared in pharmaceutical lab of department of Ras Shastra and Bhaishajya Kalpana and further subjected to analytical studies for various parameters.

All Parameters was taken according to "Protocol of testing of ASU medicines[2]" & Ayurvedic Pharmacopoeia of Indian Medicines, published by Govt. of India, Dept. of Ayush.

Most of tests were conducted at departmental analytical lab and remaining tests were conducted at S.R.LABS, Pratap Nagar, Jaipur.

Procedures

Organoleptic characters

"Organoleptic evaluation" of drugs refers to the evaluation of a drug by Colour, Odour, Size, Shape, Taste and special features including touch, texture etc. with the help of *Jnanendriya*. The obtained results were shown in table no. 1.

Physico-chemical analysis^[3]

Loss on drying (LOD)

Place 10 gm accurately weighed of grind sample (without preliminary drying) in a tarred evaporating dish. After placing the above said amount of the drug in the tarred evaporating dish, dry at 105°C for 5 hours and weigh. Continue the drying and weighing at one hour interval until difference between two successive weighing corresponds to not than 0.25 percent. Constant weight is reached when two consecutive weighing after drying and cooling for 30 minutes in a desiccators. Weight loss was calculated and expressed as % w/w. The obtained results were shown in table no. 2.

pH Determination (10% Aqueous solution)

Immerse the Digital pH meter in the solution under examination & measure the pH at the same temperature which was used for standard solutions. At the end of the set of measurements, record the pH of the solution used to standardize the meter. If the difference between this reading & the original value is greater than 0.05, the set of measurements must be repeated.

Take tablets of different pH and dissolved one tablet in 100ml of distilled water to prepare solutions of different pH 4 and 7. Switch on instrument. Leave it for some time. Take the buffer solution in the beaker and dip the digital pH meter in it. Carry out the same exercise for another buffer solution also, after washing the pH meter thoroughly with distilled water. After proper calibration of the Digital pH meter take the test sample (in 10% aqueous solution) and dip the pH meter in it. Note the value of pH. The same procedure is applied for 3 sample each time. The obtained results were shown in table no. 2.

Total ash

Incinerate 2 gm of drug accurately weighed and ground in a tarred silica dish. Keeps the crucible in a muffle-furnace at a temperature 550°C until free from carbon (white ash). Then

cool the content and weigh. Calculate the percentage of ash with reference to the air-dried drug. The obtained results were shown in table no. 2.

Acid-Insoluble ash

Transfer the ash obtained from Total Ash test in a 250 ml beaker without loss of ash and adds 100 ml of diluted hydrochloric acid. Wash the crucible with 10 ml of acid and transfer the washing to the beaker. Heat the beaker till the liquid boils. Then filter the solution and collect the insoluble matter on an ash less filter paper (Whatman no.41). Wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible. Dry on a hot plate and ignite at 600°C in a muffle furnace (until it becomes white ash). Allow the residue to cool in suitable desiccators for 30 minutes and weigh without delay. Repeat the process until constant weigh is obtained. Calculate the Acid-insoluble ash with reference to the air dried drug. The obtained results were shown in table no. 2.

Total solid content

Weighed empty flat bottomed petri dish (Evaporating Dish) (W₂). 10 g sample was taken in 50 ml in a petri dish. Weighed sample in petri dish (W_1) . Evaporate to thick extract on boiling water bath for 30 min. Add accurately weighed 1 gm of diatomite. Dry at 105 C for 3 hours in ovan. Cool in a dessicator for 30 min. Weighed it immediately (W₃). Deduct the weight of diatomite added, from the total weight of residue. The obtained results were shown in table no. 2.

Fat content

Take accurately weighed air dried plant material (W₁). Extract with petroleum ether (40-60°C) in Soxhlet apparatus until colourless appearance. Take and weigh evaporating dish (W_2) . Filter the extracted liquid in evaporating dish. Evaporate the solvent on water bath for 30 min. Dry the extract on tarred evaporating dish and weight up to constant weight (W₃). Calculated the percentage with reference to the plant material. The obtained results were shown in table no. 2.

Total sugar

Determination of factor of fehling solution

Take & Weighed 4.7523 gm pure Sugar (sucrose) in 500 ml Volumetric Flask. Add 50 ml of distilled water than add 5 ml conc. HCl & Keep for 24 hr. Neutralize with Sodium hydroxide & than volume make upto 500 ml. Take 50 ml of above solution & diluted upto 100 ml with

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distilled water. Transfer in burette. Take a mixture of Fehling A & Fehling B (5+5ml) in a conical flask. Preliminary Titration occurs Add methylene blue (2-3 drops) as a indicator. Titration with filtrate sugar solution. Brick Red precipitate find at end point of titration, note reading of burette.

Determination of total sugar

Weighed 1 gm of sample in conical flask. Add 20-30 ml 1 N HCl. Boil at Hot Plate than Cool. Neutralize with sodium carbonate and volume make up with 250 ml distilled water Filter with filter paper. Fill the filtrate in burette. Take a mixture of Fehling A & Fehling B (5+5ml) in a conical flask. Preliminary Titration occurs Add methylene blue (2-3 drops) as an indicator. Titration with filtrate sugar solution. Brick Red precipitate find at end point of titration, note reading of burette as a Titer value. The obtained results were shown in table no. 2.

Reducing sugar

Take 15-20 gm Sample in 100 ml Volumetric Flask. Add 5 ml Neutral lead acetate solution. Dilute upto 100 ml with distilled water & Sonicate, than kept for sedimentation. Take 25 ml of supernatant (Upper solution), transfer in 250 ml volumetric flask which contain 100 ml distilled water. Add slowly sodium oxalate solution in small quantity, so there is no further precipitate, make up volume upto 250 ml with distilled water. Mix well & filter through whatsman filter paper. Transfer filtrate in burette. Take a mixture of Fehling A & Fehling B (5+5ml) in a conical flask. Preliminary Titration occurs Add methylene blue (2-3 drops) as an indicator. Titration with filtrate sugar solution. Brick Red precipitate find at end point of titration, note reading of burette. The obtained results were shown in table no. 2.

Non-reducing sugar

% Non Reducing Sugar = Total Sugar - Reducing sugar.

The obtained results were shown in table no. 2.

TLC

Stationary phase: Pre-coated silica gel 60 F₂₅₄ aluminum plates

Mobile phase: Toluene: Ethyl acetate: formic acid (8: 2: 0.1)

Chamber saturation time: 20 minute.

Test Solution: 1 gm of formulation dissolved in methanol and then filter the liquid extract.

Make the volume up to 10 ml with methanol.

Visualization and Detection: 254 nm, 366 nm

Procedure

Take previously washed with methanol and dried TLC plate and fix dimension at X position and mark from base with help of pencil at 10 mm and 90 mm. and also left 15 mm from both sides of plate. Apply the test sample solution for each 20 μ l in the form of bands. Allow the solvent to be evaporated and place the plate in the saturated tank, possibly vertical and so that spots or bands are above the level of mobile phase. Close the tank and allow standing at room temperature until mobile phase ascended to the marked line. Remove the plate and dry and visualize as in UV-Vis light at 254 nm and 366 nm. The obtained results were shown in table no. 2.

RESULTS

Table no. 1: Showing results of organoleptic characters of lauha rasayana.

Sr. no.	Description	Result	
1.	Appearance	Thick Semi-Solid Paste	
2.	Colour	Dark brown to black	
3.	Odour	Characteristic	
4.	Taste	Sweet	

Table no. 2: Showing the Physico-chemical parameters of lauha rasayana.

Sr.no	Parameters	Results	Test Method
1.	Loss on drying (at 105 ^o C)	5.35 % w/w	API Part I, Vol. – VI, 2009
2.	pH (10% Aqueous Solution)	6.3 w/v	API Part I, Vol. – VI, 2009
3.	Total Ash	7.1 % w/w	API Part I, Vol. – VI, 2009
4.	Acid insoluble Ash	6.33 % w/w	API Part I, Vol. – VI, 2009
5.	Total solid content.	86.35 % w/w	API Part I, Vol. – VI, 2009
6.	Fat content	10.51 % w/w	API Part I, Vol. – VI, 2009
7.	Total sugar	46.15 % w/w	API Part I, Vol. – VI, 2009
8.	Reducing sugar	21.15 % w/w	API Part I, Vol. – VI, 2009
9.	Non-reducing sugar	25 % w/w	API Part I, Vol. – VI, 2009
10.	TLC (Thin Layer	Rf –	API Part I, Vol. – VI, 2009
	Chromatography)	366 nm –	
	Maethanolic extract –	0.15, 0.20,	
	(Toluene : Ethyl acetate :	0.23, 0.34,	
	Formic acid = $8:2:0.1$)	0.43	
		254 nm – 0.34	

DISCUSSION

Analytical study deals with the analysis of the values of some physical constants and chemical values of the prepared formulation. In present research work *Lauha Rasayana* was

tested on various preliminary parameters as well as on some of the sophisticated analytical tests. Here an attempt was made to put physico-chemical standards of *Lauha Rasayana*. Regarding Organoleptic characters, the colour of *Lauha Rasayana* is drak brown to black and it has a Characteristic odour. On the ground of Physico-chemical parameters. To estimate the moisture content sample was dried at 110° C for 5 hr. so Percentage weight Loss on drying of sample was 5.35 % w/w, PH of 10 % w/v solution of sample was 6.3 w/v, Total Ash percentage of Sample was 7.1 % w/w. Acid Insoluble Ash of sample was 6.33 % w/w. Total solid content percentage of Sample was 86.35 % w/w. Fat content percentage of Sample was 10.51 % w/w. Total sugar percentage of Sample was 46.15 % w/w. Reducing sugar percentage of Sample was 21.15 % w/w. Non-reducing sugar percentage of Sample was 25 % w/w. The result of TLC was, For Rf value of 366 nm – 0.15, 0.20, 0.23, 0.34, 0.43, for Rf value of 254 nm – 0.34.

CONCLUSION

Analytical test results of *Lauha Rasayana* were within normal limits These quality control parameters can be considered as tool for preparation, safety and efficacy of formulations. Hence, further clinical study can be helpful for evaluating the results.

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