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AN EVALUATION OF *DARVYADI LOHA* W.S.R TO ITS ANALYTICAL PARAMETERS AND PHYTOCHEMICAL CONSTITUENTS

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ABSTRACT

Iron is one of the chief metals which is available plentifully and which is very much indispensable for the normal sustenance of the body. We get various references of *Lauha Kalpanas* in classics which can be recommended as iron supplements in Iron deficiency anaemia. On analysing the method of preparation of these *Kalpanas* , one can understand that *Lauha Kalpanas* are iron containing formulations administered in a better acceptable form and with increased bioavailability.*Darvyadi Loha* is one such reference mentioned in *Rasendra Sara Sangraha* in which the main ingredients are *Daarvi, Amalakki, Vibhitaki, Haritaki, Pippali, Maricha, Shunti, Vidanga* and *Lohabhasma*. Most of the modern hematinics usually induce some adverse drug reactions whereas *Lauha Kalpana* are formulated in such a way that it has seldom any adverse drug reactions associated with it on administration. Hence, it is the need of the hour to critically analyse the formulations and with the aid of various standardisation parameters in order to comprehend the safety profile

Hence, the current study aims at standardisation of *Darvyadi Loha* as per physicochemical parameters as well as phytochemical evaluation.

Keywords: *Darvyadi Loha*, Standardization, physicochemical parameters, phytochemical evaluation, *Pandu Roga*

INTRODUCTION: *Lauha* is one of the major metals which is available copiously and which is very much vital for the normal sustenance of the body. We get various references of *Lauha Kalpanas* in classics which can be recommended as iron supplements in Iron deficiency anaemia. On analysing the method of preparation of these *Kalpanas*, one can understand that *Lauha Kalpanas* are iron containing formulations administered in a better acceptable form with increased bioavailability.

Darvyadi Loha[1] is one such reference mentioned in *Rasendra Sara Sangraha* in which the main ingredients are *Daarvi, Amalakki, Vibhitaki, Haritaki, Pippali, Maricha, Shunti, Vidanga* and *Lohabhasma*. Most of the hematinics[2] are known to induce some adverse reactions related to gastrointestinal tract such as nausea, vomiting, epigastric pain, borborygami, constipation, black faeces and diarrhea. Whereas *Lauha Kalpana* are formulated in such a way that it has seldom any adverse drug reactions associated with it on administration .Hence, it is the need of the hour to critically analyse the formulations with the aid of various standardisation parameters in order to understand the safety profile. This work can also be considered for

standardisation of *Darvyadi Loha Kalpana* as a similar study has not been conducted for *Darvyadi Loha.*

MATERIALS AND METHODS:

Objectives

1.	Physico-chemical parameters
	✓ Loss on drying at 105^0 C ✓ Total ash ✓ Acid insoluble ash ✓ Water soluble ash ✓ Alcohol soluble extractive ✓ Water soluble extractive ✓ Uniformity of weight ✓ Disintegration time
2.	Preliminary phytochemical evaluation
	✓ Test for alkaloids ✓ Test for carbohydrates ✓ Test for saponins ✓ Test for tannins ✓ Test for flavonoids ✓ Test for phenol ✓ Test for Coumarins ✓ Test for triterpenoids ✓ Test for carboxylic acid ✓ Test for resin ✓ Test for quinine

SOURCE OF DATA : The study of various physicochemical analysis and preliminary phytochemical analysis of *Darvyadi Loha* were carried out at S.D.M Centre for Research in Ayurveda and Allied Sciences, Udupi.

METHODOLOGY

The raw drugs required for the preparation of *Darvyadi Loha* were collected from the GMP certified SDM Ayurveda Pharmacy, Udupi and they were identified as genuine samples by Head, Department of Rasashastra and Bhaisajya Kalpana. Pharmaceutical Preparation of *Darvyadi Loha* as per the reference quoted in *Pandu Roga Chikitsa Prakarana* of *Rasendra Sarasangraha was* carried out in SDM Ayurveda Pharmacy, Udupi.

- **Physico-chemical evaluation of *Darvyadi Loha*:**

Loss on drying at 105^oC

10 g of sample was placed in tared evaporating dish. It was dried at 105˚C for 5 hours in hot air oven and weighed. The drying was continued until difference between two successive weights was not more than 0.01 after cooling in desiccator. Percentage of moisture was calculated with reference to weight of the sample.

Total Ash

2 g of sample was incinerated in a tared platinum crucible at temperature not exceeding 450˚C until carbon free ash is obtained. Percentage of ash was calculated with reference to weight of the sample.

Acid insoluble Ash

To the crucible containing total ash, add 25ml of dilute HCl and boil. Collect the insoluble matter on ashless filter paper (Whatmann 41) and 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 to constant weight. Allow the residue to cool in

suitable desiccator for 30 mins and weigh without delay. Calculate the content of acid insoluble ash with reference to the air-dried drug.

Water soluble ash

Boil the ash for 5 min with 25 ml of water; collect insoluble matter on an ashless filter paper, wash with hot water, and ignite for 15 min at a temperature not exceeding 450˚C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash with reference to the air-dried sample.

Alcohol soluble extractive

4 grams of sample was accurately weighed in a glass stoppered flask. Added 100 ml of distilled Alcohol (approximately 95%). Shaken occasionally for 6 hours. Allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporated to dryness on a water bath. Kept it in an air oven at 105°C for 6 hours, cool in desiccator for 30 minutes and weighed. Calculated the percentage of alcohol extractable matter of the sample. Repeated the experiment twice, and taken the average value.

Water soluble extractive:

4 grams of sample was accurately weighed in a glass stoppered flask. Add 100 ml of distilled water, shaken occasionally for 6 hours. Allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent. Pipetted out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporated to dryness on a water bath. Kept in an air oven at 105°C for 6 hours. Cooled in a desiccator and weighed. Repeated the experiment twice and took the average value.

- **Preliminary phytochemical tests[3]:**

Tests for alkaloids

- ***Dragendroff's test:*** To a few mg of extract dissolved in alcohol, a few drops of acetic acid and Dragendroff's reagent were added and shaken well. An orange red precipitate formed indicates the presence of alkaloids.

Tests for carbohydrates

- ***Molisch's test:*** To the extract, 1 ml of α-naphthol solution and conc. sulphuric acid was added along the sides of test tube. Violet colour formed at the junction of the two liquids indicates the presence of carbohydrates.

Test for saponins

To a few mg of extract, distilled water was added and shaken. Stable froth formation indicates the presence of saponin.

Test for tannins

To the extract, a few drops of dilute solution of ferric chloride was added, formation of dark blue to black colour shows the presence of tannins.

Test for flavonoids

Shinoda's test: To the extract in alcohol, a few magnesium turnings and few drops of conc. hydrochloric acid were added and heated on a water bath. Formation of red to pink colour indicates the presence of flavonoids.

Test for phenol

To the extract in alcohol, added two drops of alcoholic ferric chloride. Formation of blue to blue black indicates the presence of phenol.

Test for coumarins

To the extract in alcohol, a few drops of 2 N sodium hydroxide solution was added. Dark yellow colour formation indicates the presence of coumarins.

Test for triterpenoids

The extract was warmed with tin bits and few drops of thionyl chloride. Formation of pink colour indicates the presence of triterpenoids.

Test for carboxylic acid

Extract dissolved in water is treated with sodium bicarbonate. Brisk effervescence indicates the presence of carboxylic acid.

Test for resin

Few mg of the sample was mixed with water and acetone. Turbidity indicates the presence of resins.

Test for quinone

A few mg of alcohol extract was treated with 0.5% of sodium hydroxide. Deep coloration like pink, purple or red indicates the presence of quinone.

Uniformity of weight[3]

An intact capsule was weighed. Opened it without losing any part of the shell and removed the contents as completely as possible. For soft gelatin capsules, washed the shell with a suitable solvent and kept aside until the odour of the solvent is not perceptible. Weighed the shell. The difference between the weighing gives the weight of the contents. The procedure was repeated with another 19 capsules.

Average Weight of tablet	Percentage deviation
Less than 300mg	10
300mg or more	7.5

Disintegration time[3]

The tank of the disintegration apparatus was filled with distilled water up to the mark. 750 ml of distilled water in each of the 1000 ml beaker was taken. The timer of the instrument was set for 60 minutes. The temperature of water in beakers to 37°C and that of water in the main tank to 37.5°C was maintained. One tablet was introduced into each tube and, added a disk to each tube. The assembly was suspended in the beaker containing water and the apparatus was operated. The time duration at which the tablet disintegrated was noted.

OBSERVATIONS AND RESULTS

The results obtained after assessing the Standardization parameters (Table 1) and result of preliminary phytochemical screening of *Darvyadi Loha* (Table 2 and Table 3) are tabulated below.

Table 1: Results of standardization parameters of *Darvyadi Loha* capsules

Parameter	Results n = 3 %w/w
Loss on drying	13.77±0.01
Total Ash	8.80±0.01
Acid Insoluble Ash	6.63±0.00
Water soluble Ash	2.29±0.00
Alcohol soluble extractive value	7.22±0.01
Water soluble extractive value	12.25±0.00
Capsule weight variation	0.632±0.00
Shell wt.	0.108±0.00
Uniformity of content	0.523±0.00
Disintegration time (min: sec)	14:20

Table 2: Results of preliminary phytochemical screening of *Darvyadi Loha*

Alkaloid	+
Carbohydrate	+
Tannin	+
Flavonoids	+
Saponins	-
Terpenoid	+
Coumarins	-
Phenols	+

Carboxylic acid	+
Amino acids	-
Resin	+
Quinone	-

[(+) - present; (-) – negative]

Table 3: Tests and results of preliminary phytochemical screening of *Darvyadi Loha*

Tests	Color if positive	Alcoholic extract
Alkaloids		
Dragendroff's test	Orange red precipitate	White precipitate
Carbohydrate		
Molish test	Violet ring	Violet ring
Tannin		
With $FeCl_3$	Dark blue or green or brown	Dark blue to black color
Flavanoids		
Shinoda's test	Red or pink	Pink color
Saponins		
With water	Stable froth	No froth
Triterpenoids		
Tin and thionyl chloride test	Pink/Pinkish red color	Red color
Coumarins		
With 2 N NaOH	Yellow	Buff color
Phenols		
With alcoholic ferric chloride	Blue to blue black	Blue to blue black
Carboxylic acid		
With water and $NaHCO_3$	Brisk effervescence	No brisk effervvescence
Amino acid		
With ninhydrine reagent	Purple colour	Green color
Resin		
With aqueous acetone	Turbidity	Turbidity
Quinone		
0.5% NaOH	Pink/purple/red	Buff color

DISCUSSION: *Darvyadi Loha*[1] is described under *Pandu Roga Chikitsa Prakarana* in Ayurvedic treatises like *Rasendra Sara Sangraha, Bhaishajya Ratnavali, Chakradatta* etc. The present study dealt with the analytical parameters to determine the safety profile of the drug. Loss on Drying at 105^0 indicates the amount of water and volatile matter in a sample when it is dried under specific conditions for 3 hours. An excess of water

will encourage microbial growth, the presence of fungi or insects and deterioration following hydrolysis. Total ash method is intended to measure the total amount of material remaining after ignition. It is useful in defining the genuineness and purity of the sample. Acid insoluble ash indicates siliceous impurities. Water soluble ash gives an approximation of inorganic contents. Alcohol soluble extractive values and water-soluble extractive value is used to determine the quality and to distinguish adulteration due to exhausted or erroneously processed drugs. Uniformity of weight will ensure the even distribution of ingredients in the dosage form which has a direct impact on the therapeutic range of the drug.

Disintegration time determines whether the given sample will disintegrate within the given time in a liquid medium under experimental conditions and the result will provide data related to the drug's bioavailability. The disintegration time (min: sec) is 14:20 for the given sample which is within acceptable range. Iron absorption is inversely proportional to amount of iron present in the duodenum and jejunum while the frequency of gastrointestinal side effects is directly proportional to that amount. Slow release preparations allow only a small amount of iron at any given moment to come in direct contact with the duodenal mucosa, thus improving both absorption and gastrointestinal tolerance[5].

Phytochemical tests aim at extraction, screening and identification of the medicinally active substances found in plants. On preliminary phytochemical screening of *Darvyadi-Loha* ,the presence of Alkaloid, Carbohydrate, Tannin, Flavanoids, Terpenoid, Phenols, Carboxylic acid and Resin was inferred whereas Saponins, Coumarins, Amino acids and Quinone were inferred to be absent in the given sample.

CONCLUSION: The study reveals that quality control parameters were considered during the pharmaceutical preparation of *Darvyadi Loha*. Physicochemical analysis and phytochemical tests were conducted for standardisation aspects. It can be concluded that the similar pharmaceutical procedures can be implemented for large scale production with quality assurance. The present study can be considered as a standard for future drug reference.

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