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PHARMACEUTICAL AND ANALYTICAL STUDY OF DHATRIAVLEHA

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ABSTRACT

Avleha is one of the semi- solid forms of Ayurvedic medicine. It is prepared from herbs with addition of Jaggery or Sugar. As these products last for longer time and also they are easily palatable there is an increasing demand for *Avlehas* in the market today. Because of its sweet taste many *Avlehas* are used in pediatric practice, looking for its increasing demand the standardization i.e. setting standards for *Avleha* is a need now a days. Absence of reference standards for compound formulations is a need on the way towards standardization. The present study was aimed for pharmaceutical and analytical study of *dhatriavleha.* Analytical study provides us the objective parameters for standardization. It helps in understanding and interpretation of a drug and its structure. *Dhatriavaleha* formulation mentioned in *Charak Chikitsasthana* which is useful in *Pandu, Kamala, and Halimak* is taken for present study. *DhatriAvleha* includes *draksha, sunthi, pippali, vanshlochan, yashtimadhu, madhu, sharkara.* Three batches of *Dhatriavaleha* were prepared to develop Standard Operating Procedure. In Analytical study prepared *Dhatriavaleha* was tested for organoleptic parameters and physico-chemical parameters like loss on drying, total sugar content, alkaloid content, total ash value, acid insoluble ash value, methanol soluble extractive value, water soluble extractive value, pH value. HPLC was done. Values of Physico-chemical parameters can be taken as measures for quality control of *Dhatriavaleha.*

Keywords: *DhatriAvleha*, Analytical Study, HPLC

INTRODUCTION

Bhaishajya Kalpana is a branch of Ayurveda which involves a perfect combination of herbal drugs as well as minerals in various types of preparations. The word *Bhaishajya* means relating to medicine i.e. *Bhaishajya* and *Kalpana* means formulation. Ayurveda considers each and every herb in the universe to be a *Bhaishajya* but the thing is, it should be taken in proper quantity and combinations. Preparations in *Bhaishajya Kalpana* have more peculiarities about its quantity in any of the formulations. It mainly focuses on the quantity and quality of the drugs and thus is growing need

in today's market. The quality control and standardization facets of herbal drugs stay as difficult task even in the 21st century. Proper identification, surety, purity and analytical measures are unavoidable stages required for quality assurance and standardization of herbal formulation. Absence of reference standards for compound formulations is a hiridance on the way of standardization. Chemical Analysis ensures chemical constituents and standards of medicine, responsible for the efficacy.

Avleha is one of the formulations from *Bhaishajyakalpana* which is having high demand today. If we go through the references, the yoga of *DhatriAvleha* is found in Reference: *Charak Chikitsasthana* 16/101-102. It contains *draksha, sunthi, pippali, vanshlochan, yashtimadhu, madhu, sharkara*. The present study was aimed for analytical study of *dhatriavleha.* Analytical study provides us the objective parameters for standardization. It helps in understanding and interpretation of a drug and its structure. Study Includes Analytical study for standardization of *Dhatriavleha* was carried out on the basis of classically

Illustrated organoleptic tests and modern parameters i.e. Physico chemical properties were carried out for Loss of drying, Value of Reducing sugar, Alkaloid content, Ash value, Value of acid insoluble ash, Alcohol (Methanol) soluble extractive value. Water soluble extractive value. pH range, HPLC graph. Due to increasing demand of *Avleha Kalpana* the standards need to be set and thus the study was selected.

Aim and Objectives:

- To prepare *DhatriAvleha.*
- To compare the three batches of *Avaleha* in order to see the changes occurred.
- To carry out Physico- chemical Analysis of *Dhatri Avleha.*
- To establish standard parameters for *Dhatri Avleha.*

MATERIAL &METHOD: Reference: *Charak Chikitsasthana*16/101-102

Raw materials (*Amalaki, mannuka, pippali, shunthi, yashtimadhu. vanshlochana, madhu, Sharkara*) required to prepare *DhatriAvaleha* are collected from reliable source.

Before preparation all raw materials (*amalaki, mannuka, pippali, shunthi, yashtimadhu*) are authenticated in reliable institute and Agmark honey is taken standardization of *Vanshlochana* is done. Preparation was done thrice and observations were noted.

Ingredients:

Table 1: Showing Quantity of ingredients taken for all 3 Batches

Sr no	Contents	Ingredients	Quantity
1	*Drava –dravya*	*Amalaki swarasa*	2050 lit
2	*Madhur dravya*	*Sharkara*	400 gm
3	*Kalka dravya*	*Seedless mannukakalka*	128 gm
4	*Prakshepdravya*	*Pippali*	128 gm
		Shunthi	16 gm
		Yashtimadhu	16 gm
		Vanshlochan	16 gm
		Madhu	128 gm

1. Preparation of *amalakiswarasa* Purpose: Preparation of *drava-dravya*

Type of procedure: *Yantranishpiditad* method

Equipments: Knife, mixer, cotton cloth, stainless steel vessel, Measuring cylinder.

Ingredients: *Amalaki* fruits-4 kg

Procedure:

1) *Amalaki* fruits were procured from local market.
2) These fruits were cleaned, washed and dried.
3) 4 Kg of *Amalaki* fruits were taken.
4) Seeds were separated from fruits.
5) Fruits were grinded by using mixer.
6) Extraction of *swarasa* was done by squeezing and it was filtered through the cotton cloth.
7) *Swarasa* was collected in stainless steel vessel.

2. Avaleha preparation

Method of preparation:

1) Freshly prepared *Amalaki swarasa* was poured in stainless steel vessel.
2) Sugar was mixed to the *swarasa* and mild heat was given.
3) It was stirred continuously.
4) *Mannukakalka* was added at 98^0C before *pakvalakshana* observed and stirred properly.
5) Mild heat was given till *Pakvalakshana* observed.
6) Fine powder of *prakshepdravya* with mesh size 60 (*Pippali, shunthi, Yashtimadhu, vanshlochana*) were added at 52^0C after removing vessel from heat
7) Mixture was stirred continuously and vigorously to get homogenous mixture.
8) After cooling *Madhu* (Honey) was added at 32^0C.
9) Prepared *Dhatriavaleha* was packed in air tight dry container.

Precautions:

Continuous stirring should be needed throughout procedure. Mild heat should be maintained throughout procedure. Fine powders of *prakshepdravya* should be added. Fine powders of *prakshepdravya* should be added in small quantity and stirred continuously and vigorously to form homogenous mixture.
Same procedure is repeated for 2 times again.

EVALUATION PARAMETERS & RESULTS

Dhatriavaleha preparation.

Table 2: Showing observations during preparation of *Amalaki swarasa*.

Sr No	Observations
1	Froth was seen during the process.
2	Some amount of sediment was seen at the bottom of vessel.

Table 3: Showing results obtained during preparation of *Amalaki swarasa*.

Sr No	Results	Quantity
1	Weight of *Amalaki* fruit with seed	4 kg
2	Weight of *Amalaki* without seed	3642 gm
3	Weight of seed	358 gm
4	Weight of paste of *Amalaki* after squeezing	951 gm
5	Obtained *swarasa*	2180 ml

Table 4: Showing practical details regarding *Dhatriavaleha*.

Sr no	Parameters	Batch I	Batch II	Batch III
1	Temp. of *Amalaki swarasa*	28^0C	28^0C	28^0C
2	Temp. at which *kalka* added	98^0C	98^0C	98^0C
3	Temp. at which *Prakshepdravya*added	52^0C	52^0C	52^0C
4	Temp. at which honey added	30^0C	30^0C	30^0C

Table 5: Showing organoleptic characters of *Dhatriavaleha.*

Sr no	Organoleptic test	Observations Batch 1	Observations Batch 2	Observations Batch 3
1	Consistency	Semi solid	Semi solid	Semi solid
2	Colour	Dark brown	Dark brown	Dark brown
3	Taste	*Katu, Kashaya*	*Katu, Kashaya*	*Katu, Kashaya*
4	Smell	Characteristic	Characteristic	Characteristic

Table 6: Showing obtained yield of Avaleha.

Sr No	Results	Quantity I	Quantity II	Quantity III
1	Initial weight of Ingredients	2882 gm	2882 gm	2882 gm
2	Obtained avaleha	1105 gm	1114 gm	1150 gm

Table 7: Showing duration required to prepare.

Sr No	Results	Duration I	Duration II	Duration III
1	Time required to prepare *Amalaki swarasa*	4 hr 30 min	4 hr 15 min	4 hr 30 min
2	Time taken for *pakvalakshana* observed	4 hr 36 min.	4 hr 34 min.	4 hr 34 min.
3	Time required to prepare *avaleha*	5 hr 40 min	5 hr 30 min	5 hr 45min

Observations of *Avaleha* Showing temperature variations:

Temperature was noted from zero minutes upto 5 hours 40 minutes and more the first temperature noted was 28^0c and it gradually increased up to 100^0 c and then gradually decreased after the heating was stopped and honey was added at 30^0 c

***Siddhi Lakshana* observed:**

Tantumattvam: Thread like appearance.

Appsumajjati: It is settled down in water.

Piditemudra: When it is pressed between two fingers then impression of fingers appear over it.

Ishtagandhavarna rasa utpatti– It has appropriate smell, colour and taste.

Physico chemical analysis was carried out with ancient and modern parameters.

A. Classical parameters:

The ancient Parameters like *Varna, gandha* etc. were carried out for *Dhatri Avleha*.in *Rasashastra* and *Bhaishajya Kalpana* Dept details are mentioned in table 1

Showing organoleptic parameters of all three batches.

Sr no	Parameters	Batch I	Batch II	Batch III
1	Consistency	Semi solid	Semi solid	Semi solid
2	Colour	Dark brown	Dark brown	Dark brown
3	Taste	*Katu, Kashaya*	*Katu, Kashaya*	*Katu, Kashaya*
4	Smell	Characteristic	Characteristic	Characteristic

Physico-Chemical Tests:

Physiochemical tests like Loss of drying, Value of Reducing sugar, Alkaloid content, Ash value, Value of acid insoluble ash, Alcohol (Methanol) soluble extractive value. Water soluble extractive value. pH range, HPLC graph.

Modern parameters:

B. Physical tests

1. Organoleptic characters Colour, smell, taste of the given sample was tested using sensory organs and the same were noted in table no 1.

1) Loss on Drying: **Aim**: This was conducted to find out the moisture content in the sample. **Principle**: The amount of moisture and other

substance that get volatilized in the sample, which is specific to drug or formulation and in controlled heating of sample for specific duration removes the components and a constant weight is obtained .The loss is observed in the initial weight and is expressed as LOD percentage w/w**. Procedure** : 5 gm sample was taken in a previously dried and weighed evaporating dish and dried initially on a water bath and finally in an oven at 105°C temperature till constant weight is obtained. From the weights noted, the loss on drying of the sample was calculated and expressed as percentage w/w.

2) Alkaloid Content: **Aim:** This test was carried out for presence of alkaloid content. **Procedure:** A portion of the methanol extract was taken in watch glass, the solvent evaporated, to it few drops of dil. HCL followed by few drops of Dragendro's reagent was added. Formation of orange colored precipitate indicates the presence of alkaloid.

3) Total Ash:- **Aim :** This test was done to assess the total ash value of sample. **Procedure:** 3 gm of accurately weighted sample taken in crucible and was incinerate at a temperature not exceeding 4500 until free from carbon. Allow cooling and weighted. Carbon free ash could not obtained in this way therefore charred mass exhausted with hot water and residue was collected on an ash less filter paper. Residue and filter paper were incinerated filtrate was added evaporated to dryness and ignited at a temperature not exceeding 4500.Value of total ash was calculated with reference to air-dried drug.

4) Acid insoluble ash:-Aim: This test was done to assess the acid insoluble ash value of sample. **Procedure:** Dilute hydrochloric acid was added in crucible which contains ash. Solution was filtered and insoluble matter was collected on an ash less filter paper (whatman41). It was washed with hot water until the filtrate gets neutral. Filter paper containing the insoluble matter transferred to the original crucible. It is dried on hot plate and ignited to constant weight. It was allowed to cool in desiccators for 30 minutes and weighted without delay. Value of acid insoluble ash was calculated with Reference to air-dried drug.

5) Water soluble extractive:- **Aim:** Estimation of Water soluble extractive value of sample. Procedure: 5 gm *Avaleha* sample was taken in a clean conical flask; 100 ml water was added to it and shaken repeatedly till it mixed properly. Flasks were closed and kept overnight. Next day they were filtered and 20 ml filtrate was taken in an evaporating dish, which was clean, dry and previously weighed. Then the samples were evaporated on water bath, dried in oven at 110 °C till constant weight, cooled and weighed. From the weight of the residue obtained the percentage of water soluble extractive was calculated.

6) Alcohol Soluble Extractive:-Aim: Estimation of alcohol soluble extractive value of sample **Procedure:** Methanol soluble extractive of the samples of was determined in the similar way like water soluble extractive but using methanol as solvent instead of water.

7) Total Sugar Content:-Aim: Estimation of total, reducing and non-reducing sugars. **Procedure:** Preparation of solution for analysis: 2 gm sample of *DhatriAvaleha* was taken in 100 ml beaker and 20ml distilled water was added to it and boiled it for approximate for 1 minute on hot plate. Filter it by cotton & wash it by distilled water. To the filtrate 2 to5 ml of lead acetate was added & is heated. Then it is filtered by cotton &simple filter paper & washed by distilled water & volume should not be more than 200 ml. Then it was filtered. To the filtrate sodium oxalate was added to dissolve excess lead acetate and to get a clear solution. This solution was filtered through the filter bed made up of glass wool, cotton and Whattman no. 1 filter paper to get a clear solution, washing was given by distilled water and the volume was made up to 250 ml.

Determination of Reducing sugar: To 10 ml. of above solution, 20 ml. each of Fehling's A and Fehling's B solutions were added, boiled for three minutes and filtered through filter bed (glass wool,

cotton and Whattman no.1 filter paper). Repeated washing was given by hot distilled water till clear, colorless filtrate was obtained. Precipitate of cuprous oxide (residue) was then taken with acid ferric solution to dissolve the precipitate completely in it. This solution was titrated against 0.1 N KMnO4 solution using orthophen anthrolin as indicator. At the end point the color changes to green. From the amount of KMnO4 solution required the amount of copper was calculated. Then percentage of sugar content was determined from Hammond table.

Determination of Total Sugar: 10 ml of the clarified solution was taken; to it 5 ml of 6N HCL was added and heated on water bath at 69-71°C. This is then treated with diluted NaOH solution by using phenolphthalein as indicator till pink color appeared. Volume was made up to 100 ml. For the determination of total sugar 10 ml of this solution was taken and the remaining procedure is same as that of reducing sugar. Percentage of total sugar was calculated from Hammond table.

Determination of non-reducing sugar: The non-reducing sugar content was obtained by subtracting reducing sugar from total sugar. Non reducing sugar = Total sugar – Reducing sugar.

8) pH:-The pH value of an aqueous liquid may be defined as the common logarithm of the reciprocal of the hydrogen ion concentration expressed in g per liter. **Aim:** Although this definition provides a useful practical means for the quantitative indication of the acidity or alkalinity of a solution.

HPLC Definition: It is now major chromatographic method used for therapeutic drug monitoring. It is also known as high pressure liquid chromatography, is essentially a form of column chromatography in which the stationary phase consists of small particles (3-50µm) packing contained in a column with a small bore (2-5mm), one end of which is attached to a source of pressurized liquid eluent (mobile phase). In HPLC, the mobile phased is forced through the column under High pressure. The three forms of high performance liquid chromatography most often used are ion exchange, partition and adsorption. HPLC can be used to analyze almost all the compounds in the herbal medicines. Reversed-phase (RP) columns may be the most popular columns used in the analytical separation of herbal medicines. It has most versatile, safest, dependable, fastest and sensitive chromatographic technique for the quality control of drug component. Qualities and quantitative analysis of drug material was done by this method. Preparation of sample: Dissolve about 10 mg of sample in methanol in a 10-ml volumetric flask and make up the volume. Prepare test solution of 100µg/ml by suitable dilution from stock solution.

RESULTS:

1) Loss on drying Table 2 Showing results of loss on drying.

Sr. No	*Dhatriavaleha*	Loss on drying 105oC
1.	Batch I	4.9 %
2.	Batch II	4.1%
3.	Batch III	4.3 %

There is slight variation in Batch II and Batch III from 4.1% to 4.3%.In batch I it is 4.9%.

2) Sugar content Reducing sugar Table 3 Showing results of reducing sugar.

Sr. No.	*Dhatriavaleha*	Reducing sugar
1.	Batch I	38%
2.	Batch II	39%
3.	Batch III	37%

Value of reducing sugar is ranged in between 37-39% for all batches.

Non Reducing sugar Table 4 Showing results of non reducing sugar.

Sr. No.	*Dhatriavaleha*	Non Reducing sugar
1.	Batch I	5%
2.	Batch II	5%
3.	Batch III	6%

There is slight variation in Non reducing sugar of all batches. Its value is 5% same for Batch I and II.In Batch III it is 6%.

Total sugar Table 5 Showing results of total sugar.

Sr. No	*Dhatriavaleha*	. Total sugar
1.	Batch I	43%
2.	Batch II	44%
3.	Batch III	43%

Value of total sugar is same in batch I and III.In batch II it is 44%.which is negligible

3) Alkaloid content Table 6 Showing results of alkaloid content.

Sr. No.	*Dhatriavaleha*	Alkaloid content
1.	Batch I	Present
2.	Batch II	Present
3.	Batch III	Present

Alkaloid content is present in all batches.

4) Ash value Table 7 Showing results of ash value.

Sr. No.	*Dhatriavaleha*	Ash value (not more than 2%)
1.	Batch I	1.6%
2.	Batch II	1.6%
3.	Batch III	1.5%

There is slight variation in ash value of all batches. Its value is 1.6% which is same in Batch I and II.In Batch III it is 1.5% .

5) Acid insoluble ash Table 8 Showing results of acid insoluble ash.

Sr. No.	*Dhatriavaleha*	Acid insoluble ash(not more than 0.8%)
1.	Batch I	0.3%
2.	Batch II	0.4%
3.	Batch III	0.4%

Value of acid insoluble ash is 0.4% in batch II and III.In batch I its value is 0.3%.

6) Alcohol soluble extractive value Table 9 Showing results of alcoholsoluble extractive value.

Sr. No.	*Dhatriavaleha*	Alcohol soluble extractive value (not less than 55 %)
1.	Batch I	59%
2.	Batch II	60%
3.	Batch III	61%

Alcohol (Methanol) soluble extractive value in all batch ranged in between 59%-61%.

7) Water soluble extractive value Table 10 Showing results of water soluble extractive value.

Sr. No.	*Dhatriavaleha*	Water soluble extractive value (not less than 65%)
1.	Batch I	79%
2.	Batch II	77.3%
3.	Batch III	76%

Batch Water soluble extractive value in all batch ranged in between 76%-79%.

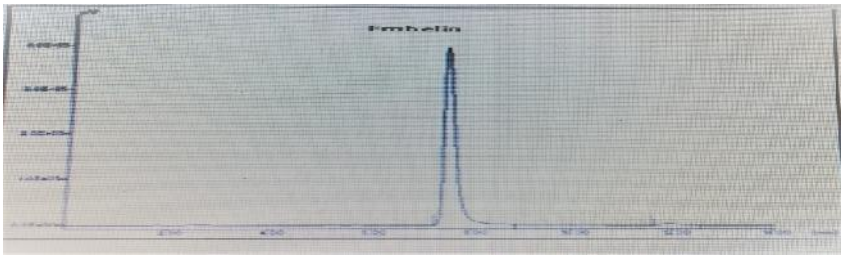
8) pH Table 11 Showing results of pH value.

Sr. No.	*Dhatriavaleha*	pH
1.	Batch I	4.71
2.	Batch II	4.67
3.	Batch III	4.70

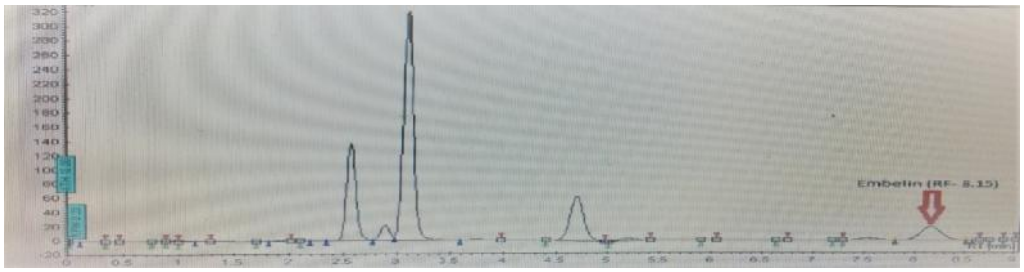
pH of three batches ranged in between 4.67-4.71, which is acidic.

9) HPLC Analysis

A) HPLC analysis of Embelin standard Graph 1 Showing HPLC analysis of Emelin standard.



B) HPLC analysis of Embelin in Dhatriavaleha Graph 2 Showing HPLC analysis of Emelinin*Dhatriavaleha.*



DISCUSSION

Analytical Study: Analysis of finished product with the suitable parameters is an essential component of drug standardization. Standardization is an essential measurement for ensuring the quality control of the herbal drugs. Quality control parameters are necessary to establish safety, efficacy. The parameters used for analysis are organolepetic characters, physico-chemical parameters and HPLC. Any change in raw material and method of preparation can cause variation in these parameters.

A) Organolepetic parameter: These are basic test for finished product. It includes colour, consistency, taste and smell. These tests were same for all batches.

B) Physico-chemical parameters: It includes pH, Loss on drying, total solid content, Alkaloid content, Ash value, Acid insoluble ash value, Alcohol soluble extractive, Water soluble extractive.

1) Loss on Drying: It indicates the moisture content. Higher moisture content may enhance the rate of rancidity.
2) Sugar content: It includes total sugar, reducing Sugar, non reducing Sugar.
3) Alkaloid content: Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms.
4) Total Ash: It important to illustrate the quality as well as purity of formulation. It helps in determining both physiological ash (plant tissue) and non physiological ash (extraneous matter like sand and soil).
5) Acid Insoluble Ash: It important to illustrate the quality as well as purity of formulation.
6) Water Soluble Extractive Value: It indicate the amount of active constituent extracted in aqueous solution expressed as percentage of extractive value. Less extractive value indicates the addition of exhausted material, adulteration or incorrect processing during drying storage or formulating.
7) Methanol Soluble Extractive Value: It indicates the amount of active constituent extracted in methanol expressed as percentage of extractive value. Less extractive value indicates the addition of exhausted material, adulteration or incorrect processing during drying storage or formulating.
8) pH value: pH value indicate acidity or alkalinity. It is the negative logarithm of hydrogen ions in the solution. pH of three batches is acidic. It may be due to *Amalakiswarasa* and chemical constituent present in formulation.
9) HPLC analysis- - HPLC can be used to analyze almost all the compounds in the herbal medicines. Reversed-phase (RP) columns may be the most popular columns used in the analytical separation of herbal medicines. - It can be considered HPLC graph for *Dhatriavaleha.* The retention time of Embelin standard and Embelin in *Dhatriavaleha* is 8.15.HPLC was carried out for the detection of presence of embelin in prepared *Dhatriavaleha*. As per HPLC analysis Embelin is present in *Dhatriavaleha.*

CONCLUSION

Analytical study for standardization of *Dhatriavleha* was carried out on the basis of classically illustrated organoleptic tests and modern parameters i.e. Physico chemical properties were carried out where there is slight variation from 4.1% to 4.9%. 4.3% for loss of drying. Value of reducing sugar is ranged in between 37-39%. Alkaloid content is present. There is slight variation in ash value. Its value is 1.5% to 1.6%.Value of acid insoluble ash is 0.3% to 0.4% Alcohol (Methanol) soluble extractive value ranged in between 59%-61%. Water soluble extractive value ranged in between 76%-79%. pH ranged in between 4.67-4.71, which is acidic. HPLC graph shows the Embelin Standards and it is 8.15.

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