

## PHARMACEUTICAL STANDARDISATION OF DAAMESHWARABHARA RAS:- AN AYURVEDIC HERO- MINERAL FORMULATION

Dr. Madhu Jangid\*<sup>1</sup> Dr. Avadhesh Bhatt<sup>2</sup> and Dr. Babulal Saini<sup>3</sup>

<sup>1</sup>M.D. (Ayu) Scholar, Department of Ras Shastra & Bhaishajya Kalpana, MMM Government Ayurvedic College, Udaipur.

<sup>2</sup>Professor & HOD, Department of Ras Shastra & Bhaishajya Kalpana, MMM Government Ayurvedic College, Udaipur.

<sup>3</sup>Associate Professor, Department of Ras Shastra & Bhaishajya Kalpana, MMM Government Ayurvedic College, Udaipur, Rajasthan.

**Corresponding Author: Dr. Madhu Jangid**

M.D. (Ayu) Scholar, Department of Ras Shastra & Bhaishajya Kalpana, MMM Government Ayurvedic College, Udaipur.

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### ABSTRACT

DA is an *Ayurvedic* herb mineral compound formulation mentioned in *Bhaishjya Ratnawali* and *Bharat Bhaishjya Ratnakar* in "*Hikkaswasa Rogadhikar*". In today era standardization is utmost necessary to confirm its identity and to determine its quality, purity, safety, effectiveness and acceptability of the product. The present study was executed to establish a fingerprint for this unique formulation, which can be adopted by the Ayurvedic pharmacies for drug standardisation. DA is a formulation prepared by as per standard operating procedures mentioned in classical text then it characterize by modern analytical techniques. DA main ingredient is Abharak Bhasma and nine ingredients sequentially used as *Bhavana Dravya* like *Bharangi*, *Datura*, *Giloya*, *Vasa*, *Kasmard*, *Vannimb (Bakayan)*, *Chavya*, *Pippali*, *Chitraka*. To ensure the proper preparation of main ingredient AB *Ayurvedic* classical test were used and analyzed using by modern parameter like Physio chemical test, element assay, XRD. After *Bhamsa* compiles these tests, DA was prepared and subjected for qualitative analysis like Physio- chemical analysis, HPTLC, microbial estimation and element essay. Element essay of DA and AB reveals that it contain Al, K, Fe, Mg, silica., XRD study was performed on AB that indicates AB contains ferric oxide, ferrous oxide, Alumina, Magnesium oxide and silica. Microbiological Analysis showed the total plate count was 140 cfu/g and total yeast & mold count was 40 cfu/g within specified limit as per API. HPTLC revealed presence of organic constituents from plant material. This is the first study establishing the characterization of DA.

**KEYWORD:** *Bhasma*, XRD, element analysis, hptlc.

### INTRODUCTION

Ayurveda employs the usages of *Rasoushdhi* (herbo – mineral) preparation since centuries for a wide range of maladies. Modern medicine has very less documentation regarding the therapeutic utility of metals and minerals. They are apprehensive about the safety of these compounds. The role of metals in curing ailments was first realized in Ayurveda.<sup>[1]</sup> Ancient Acharyas were conscious about the ill effect of the metals and they were well-versed in transforming them into safe effective medicine. In earlier days, the quality of medicine was not subjected for critique, but it was based on the sacred trust which existed between the physician and the patient. The technological development and apprehensions of modern science obligated the patients and physicians to be watchful about the quality assurance, safety and efficacy of the medicine. Ayurvedic medicine have no exception in the regard. Hence it is the need of the hour to produce

fingerprint for quality medicines. Many researchers have analyzed the metal and mineral based individual Bhasma. But the independent Bhasma are used occasionally. Hence it is required to develop fingerprint not only for AB bhasma but also for the compound DA formulation. DA has been mentioned in *Bhaishjya Ratnawali* and *Bharat bhaishjya Ratnakar* in "*Hikkaswasa Rogadhikar*".<sup>[2]</sup> It has other indication in *kasa*, *udarvikar*, *pandu*, *gulma*, *liver*, *galroga*, *shotha*, *Netra*, *mukh roga*, *yakshma*, *sula*, *spleen*, *fever*, *galganda*, *vomiting*, *bhrama* and *daha*. Hence this formulation was selected for clinical study of *Tamara Swara w.s.r. Bronchial asthma*. But till date no scientific work has been carried out on this formulation with respect to physiological characterization, quality test (XRD, HPTLC test, microbial test, element assay) which is essential for drug standardization.

## AIMS AND OBJECTIVES

1. To Prepare Daameshwarabhra Ras and Efficient SOP's & SMPs.
2. To Prepare Daameshwarabhra Ras as Per Reference *Bhaishjya Ratnawali*

## MATERIAL AND METHODS

Abhrak Bhasma was taken in one part. This was triturated sequentially with equal quantity of each nine bhavana dravya swarasa or kwatha (like Bharangi, Datura, Giloya, Vasa, Kasmard, Vannimb (Bakayan), Chavya, Pippali, Chitraka) separately in endrunner. (table-1) For that fresh swarasa and kwatha was taken

every time. For it 8 parts of water was added. It was kept for over night then next day it was boiled to prepare *ashtam ansavshoshitam* (1/8<sup>th</sup> reduced)<sup>[3]</sup> after every bhavana dry at room temperature after that drying of last bhavana done fine powder then capsule feeling were manufactured each capsule weight 250mg. For preparing of AB, the guidelines was adopted from various classical texts of Rasshastra and expert opinion. (tablet-2)

**Methods:-** Preparation of the “ Daameshwarabhra RAS” include following step:

1. Preparation of ‘Abhraka Bhasma’
2. Preparation of ‘Daameshwarabhra RAS’

**Table 1: Ingredients of Daameshwarabhra Rasa.**

| S. No.                       | Ingredients               | Botanical name        | Part use  | Quantity |
|------------------------------|---------------------------|-----------------------|---|----------|
| 1.                           | <i>Krishnavajrabhraka</i> | -                     |   | 1 Part   |
| Use As <i>Bhavana Dravya</i> |                           |                       |   |          |
| 2.                           | <i>Bharngi</i>            | Clerodendrom Serratum | Root, Leaf                                      | 1 Part   |
| 3.                           | <i>Dhatura</i>            | Datura Metel          | Whole Plant                                     | 1 Part   |
| 4.                           | <i>Guduchi</i>            | Tinospora Cardifolia  | Root, Stem, Leaf                                | 1 Part   |
| 5.                           | <i>Vasa</i>               | Adhatoda Vasica       | <i>Panchanga</i>                                | 1 Part   |
| 6.                           | <i>Kasmard</i>            | Cassia Occidentalis   | Root, Leaves, Seeds                             | 1 Part   |
| 7.                           | <i>Vannimb(Bakayan)</i>   | Melia Azedarach       | Leaf, Root, Bark, Wood, Stem Bark, Flower, Seed | 1 Part   |
| 8.                           | <i>Chavya</i>             | Piper Reterofraction  | Fruit, Root                                     | 1 Part   |
| 9.                           | <i>Pipplimool</i>         | Piper Longam          | Root,Fruit                                      | 1 Part   |
| 10.                          | <i>Chitrakmool</i>        | Pulmbago Zeylanica    | Root , Root Bark                                | 1 Part   |

**Table 2: Used ingredients For ABHRAKA Bhasma Prepration.**

| Procedure Name                            | Drug  |
|---|---|
| For <i>Shodhan</i> <sup>[4]</sup>         | <i>Triphala –Haritaki</i> (Terminalia Chebula), <i>Bibhitaki</i> (Terminalia Bellirica), <i>Amalaki</i> (Embllica Officinalis)  |
| For <i>Dhanyabhrikaran</i> <sup>[5]</sup> | <i>Dhanya, Kanji</i>  |
| For <i>Marana</i> <sup>[6]</sup>          | <i>Erand Patra</i> (Ricinus Cummunis), <i>Vata</i> (Ficus Benghalensis)<br><i>Kantakari</i> (Solanum Xanthocarpum), <i>VASA</i> (Adhatoda Vasica), <i>Bibhitaki</i> (TerminaliaBellirica) |
| For <i>Amritikarna</i> <sup>[7]</sup>     | <i>Triphala, Cow Ghrita</i>   |
| For <i>Lohitikarana</i> <sup>[8]</sup>    | <i>Gangeruki</i> (Grewia populifolia), (Cyperus rotundus), <i>Vata</i> (Ficus Benghalensis),<br><i>Haridra</i> (Curcuma longa), <i>Manjistha</i> (Rubia cordifolia)                       |

## Physio - Chemical Analysis

As *Abhraka Bhasma* is main ingredient of *Daameshwarabhra RAS* I went for analytical study of *Abhraka Bhasma* and at last the final product *Daameshwarabhra Ras*. The tests were conducted at **SR Testing Laboratory, Jaipur** Following test were conducted Parameters are taken according to “Protocol For Testing Of Ayurvedic, Siddha & Unani Medicines”, Dept.Of Ayush, ministry of health and family welfare, Pharmacopoeial Laboratory For Indian Medicines, Ghaziabad and **API** published by Government Of India, Department of Ayush, Ministry of Health and Family Welfare, **CCRAS**. Physiochemical analysis is the important characteristics to evaluate the quality, standardisation and safety of *Ayurvedic* drugs and provide information about correct identification and authentication of the raw drugs and formulations and

may be help in preventing it's adulteration. Determination of pH means the quantitative indication of the acidity or basic nature of a solution. Determination of loss on drying test was conducted to find out the moisture content of the drug, higher moisture content in sample show that it may decrease stability. Particle size analysis is part of particle science which represents the diameter of the particles of a powder. Determination of Total Ash is a quantity analysis technique for determining siliceous material and inorganic substance in sample. Acid Insoluble Ash shows siliceous material and heavy metals. Water Soluble Ash shows quantity of water inorganic Substance. Determination of Extractive values gravimetric analysis (Maceration Process), the extraction of any crude drug with a particular solvent yields a solution containing different phyto-constituents. The composition of these phyto-constituents in that

particular solvent depends upon the nature of the drug and solvent used.

**Table 3: Physical Parameter Of Abhraka Bhasma & Daameshwarabhra Ras.**<sup>[9]</sup>

| Parameter     | Abhraka Bhasma | Daameshwarabhra Ras |
|---------------|----------------|---------------------|
| 1. Appearance | Fine powder    | Fine powder         |
| 2. Color      | Reddish brown  | Reddish brown       |
| 3. Odour      | Characteristic | Characteristic      |
| 4. Taste      | -              | -                   |

**Table 4: Ayurvedic Classical Test of Abhraka Bhasma.**

| Parameters   | Observation of Abhraka Bhasma |
|--|-------------------------------|
| <i>Nishchandrica</i> (free from glittering particales)                                 | Complies                      |
| <i>Rekha purnatva</i> (should enter furrows of finger)                                 | Complies                      |
| <i>Varitara</i> (should float on still water)  | Complies                      |
| <i>Niswadu</i> ( tasteless)  | Complies                      |
| <i>Unnam</i> (Grains of rice can be loaded on the <i>Bhasma</i> floating on the water) | Complies                      |
| <i>Apunarbhav</i> (should not regain its initial lusture)                              | Complies                      |

**Table 5: Physico – Chemical Parameter Abhraka Bhasma & Daameshwarabhra Ras.**<sup>[10,11,12]</sup>

| Parameter                  | Abhraka Bhasma | Daameshwarabhra Ras |
|----------------------------|----------------|---------------------|
| pH Value                   | 7.39           | 6.70                |
| Loss On Drying             | 1.14 % w/w     | 1.18 % w/w          |
| Particle Size (by mesh)    | Fine           | Fine                |
| Total Ash                  | 98.56 % w/w    | 71.99 % w/w         |
| Acid Insoluble Ash         | 88.78 % w/w    | 54.29 % w/w         |
| Water Soluble Ash          | 2.70 % w/w     | 3.05 % w/w          |
| Sulphated Ash              | -              | 81.94 % w/w         |
| Water Soluble extractive   | -              | 19.38 % w/w         |
| Alcohol Soluble extractive | -              | 28.27 % w/w         |

**Table 6: Quantitative Estimation In Abhraka Bhasma & Daameshwarabhra Ras.**<sup>[13,14,15,16]</sup>

| Sample                     | Aluminum (as Al) %w/w | Silica (as Si) %w/w | Potassium (as K) %w/w | Iron (as Fe) %w/w | Magnesium (as Mg) %w/w |
|----------------------------|-----------------------|---------------------|-----------------------|-------------------|------------------------|
| <i>Abhraka Bhasma</i>      | 4.77                  | 11.30               | 6.96                  | 10.87             | 3.48                   |
| <i>Daameshwarabhra Ras</i> | 2.65                  | 9.66                | 4.67                  | 5.67              | 2.09                   |

**Table 7: Microbial Analysis for Final Product Daameshwarabhra Ras.**

| Microbial Analysis       | Limit        | Result    |
|--------------------------|--------------|-----------|
| Total plate count        | 100000 cfu/g | 140 cfu/g |
| Total yeast & mold count | 1000/g       | 40 fu/g   |

- HPTLC done for Daameshwarabhra Ras
- XRD done for *Abhraka Bhasma*

#### HPTLC analysis

**Sample preparation-**Take 2 gm powder each of tree batches of DA sample then dried the sample then dissolved in methanol and then filter the liquid extract. Make the volume up to 10 ml with methanol. 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 10 µl, 15 µl & 20 µl in the form of bands with the programming of Linomats applicator. 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 to stand 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, 366 nm & White Light. Prepare the scanning programme for completely dried plate at wavelength of 254 nm and another program to scan at 366 nm. Derivatize the plate with chemical reagents by dipping method. Dry the plate for 10 mins at 105°C & Visualize the bands at white light, also take SNAPS. Now scan derivatized plate at 510 or 450 nm & generate the scanning chromatogram. Procure the report

file containing the images and graphical data of the scanning chromatograms. The HPTLC profile along with the Rf values are shown in the mentioned (fig.-2).

**X-ray diffraction study**

X- ray diffraction techniques was performed to reveal information about the crystallographic structure,

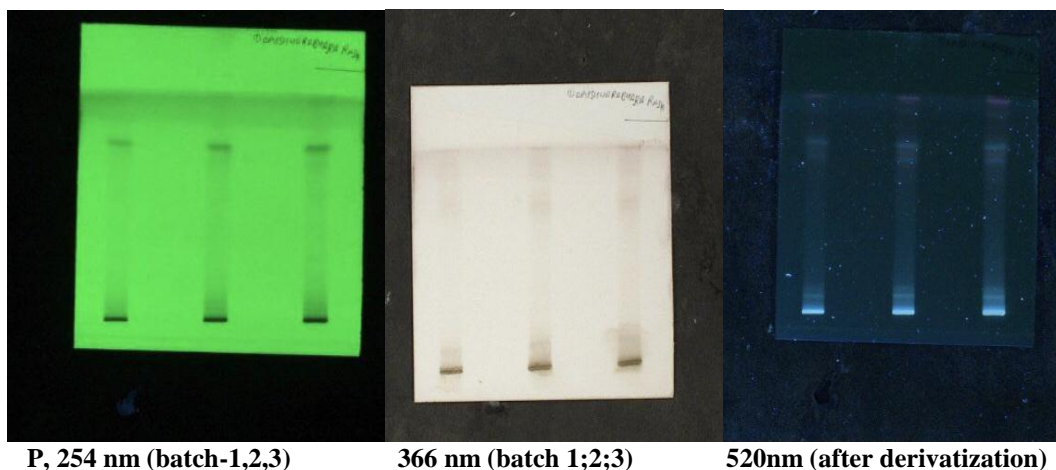
chemical composition and physical properties of materials. various peaks were observed in XRD which shows chemical composition in the bhasma. the detailed XRD data and XRD pattern are given in the below mentioned (table-8 fig-3)

**Interpretation=For Abhrak Bhasma**

**Table 8: The main compound diffraction peaks corresponding with d spacing as below mentioned compounds.**

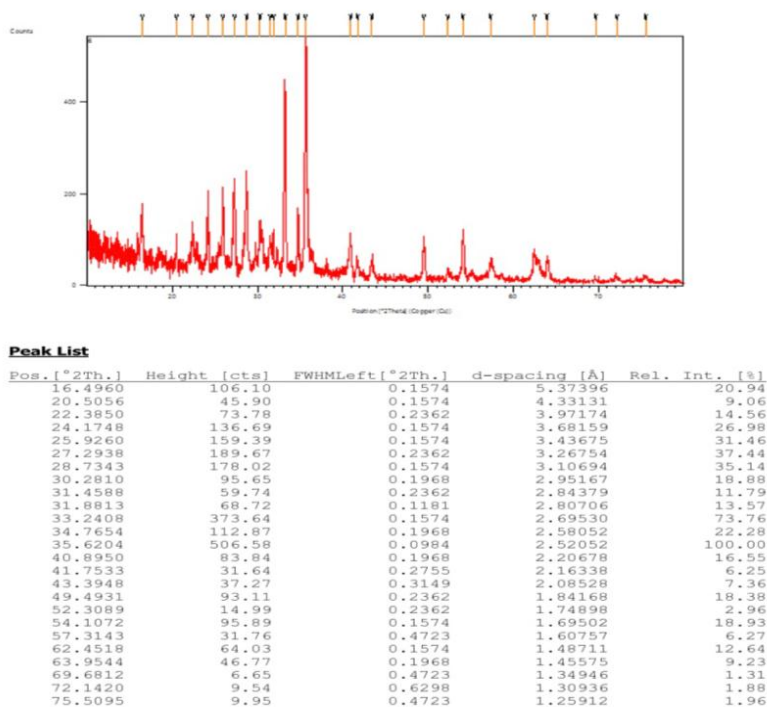
| S. no. | Compound name   | 2 <sup>θ</sup> Value | d spacing |
|--------|-----------------|----------------------|-----------|
| 1      | Ferric oxide    | 35.6859              | 2.516     |
| 2      | Ferrous oxide   | 33.2133              | 2.697     |
| 3      | Alumina         | 27.3167              | 3.264     |
| 4      | Magnesium oxide | 25.9175              | 3.437     |
| 5      | Silica          | 28.3615              | 3.146     |

Sharp single peaks shows that major compounds of Iron, Aluminium, Silica and Magnesium.



P, 254 nm (batch-1,2,3)      366 nm (batch 1;2;3)      520nm (after derivatization)

**Fig. 2: HPTLC profile of Daameshwarabhra Ras.**



**Fig. 3: XRD pattern of main ingredient Abhakra Bhasma.**

## DISCUSSION

As analytical tests decide the safety and clinical efficacy of the formulations, various parameters for analytical test were selected. As *Abhraka Bhasma* is main ingredient of **DA** so I went for analytical study of *Abhraka bhasma* and the final product **Daameshwarabhra Ras**. The tests were conducted at SR Testing Laboratory, Jaipur.

We can say **DA Ras** is a specific type of *Abhraka Bhasma* formed after bhavana of nine dravya as mentioned but big changes in some physicochemical properties and elemental assay were found before and after completion of *bhavana* as shown in table-6.

Most amazing thing I found that none of nine bhavana dravya posses *amla ras* but AB which was basic in nature became acidic after completion of bhavana showing that *amla ras* is nothing to do with acidic property. Decrease in percentage of total ash indicates bhavana increases organic contents of the formulation Decrease in percentage of acid insoluble ash denotes its increase acid soluble contents which may positively affect absorption

of **Daameshwarabhra Ras** as shown in table-5. Mentioned data reveals that after completion of bhavana elemental assay decreases significantly which may indicates that swarasa or kwatha of bhavana dravya does not contain significant amount of inorganic components. XRD study was performed on AB showed various peaks crystallographic structure, chemical composition and physical properties of materials. Sharp single peaks as shown in analytical reports shows major compounds of Iron, Aluminium, Silica and Magnesium. Microbial Analysis - The result of Microbiological Analysis showed the total plate count was 140 cfu/g and total yeast & mold count was 40 cfu/g within specified limit as per API.

When derivatized HPTLC plates prepared by three test sample solution (10ul, 15ul & 20 ul) of **Daameshwarabhra Ras** are visualized and scanned in UV at 254nm, 366nm and 510nm to generate the scanning chromatogram showed some peak. Number of peak represent presence of various ingredients in sample.

| Sr. no. | Sample                     | UV Wavelength | No. of peaks in 3 sample |      |      |
|---------|----------------------------|---------------|--------------------------|------|------|
|         |                            |               | 10ul                     | 15ul | 20ul |
| 1.      | <b>Daameshwarabhra Ras</b> | 254 nm        | 10                       | 9    | 11   |
| 2.      | <b>Daameshwarabhra Ras</b> | 366 nm        | 19                       | 19   | 18   |
| 3.      | <b>Daameshwarabhra Ras</b> | 510 nm        | 8                        | 9    | 7    |

## CONCLUSION

If classical procedures are followed as it is then we find all modern analytical parameters within normal range. the ancient classical pharmaceutical processes is safe and very effective in converting macro elements into therapeutically effective medicines of micro form. The medicine was very well tolerated by the patients no side effects and the toxicity effect were reported by any patient suggesting that the **Daameshwarabhra Ras** is absolutely safe for the internal use. Various formulation are described in various Ayurvedic texts. SOPs generation for each formulation is not possible so standardization and analysis is not available in market to give them base as per modern parameter. We sincerely hope and wish that the present study should always be pioneer as an ideal research work for coming generation.

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