A PHARMACEUTICO–ANALYTICAL STANDARDIZATION OF DURJALAJETA RASA PREPARED BY CLASSICAL METHOD

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ABSTRACT

Durjalajeta Rasa is a Herbo-mineral preparation mentioned in Rasa Chandamshu. It is one of the khalviyarasayana. The main ingredients are Shuddha vatsanabha (Aconitum ferox Wall.), Kapardika Bhasma (Cypraea moneta Linn.) and Maricha (Piper nigrum Linn.) The aim is to prepare Durjalajeta Rasa according to classical method mentioned in Rasa Chandamshu with due importance to Standard Operative Procedure and to carry out physico-chemical analysis of three different samples of Durjalajeta Rasa and to standardize the same. Durjalajeta Rasa is prepared by grinding the fine powders of the Shuddha Vatsanabha, Kapardika Bhasma and Maricha in the ratio 2:5:9 respectively with ardara swarasas for 6 hours and final product obtained in the form of Vati (pills) form. Analytical study of final product is carried out by basic quality control parameters such as average weight, uniformity of weight of pills, disintegration test, thin layer chromatography, and subjected for advanced instrumental methods of analysis like XRD, SEM-EDAX, ICPMS and HPTLC and it is observed that sample DJR1, DJR2, DJR3 are spherical, rough, grey coloured and hard in consistency. The uniformity of weight of pills, hardness, disintegration, friability of samples of Durjalajeta Rasa is approximately similar. EDAX and ICPMS showed presence of carbon, oxygen, calcium, magnesium, sulphur, potassium, chlorine, iron and copper. All major peaks observed in all sample indicate calcite as the major phase. The three samples of Durjalajeta Rasa prepared showed almost similar results in all the analysis carried out.

Keywords: Durjalajeta Rasa, Disintegration, X-Ray Diffraction, Thin layer chromatography

INTRODUCTION

Rasa shastra and Bhaishajya Kalpana are become integral part of Ayurveda. The formulations which are prepared by using Parada as a chief ingredient are considered to be as Rasas ushadvhies. This Rasas ushadvhies are very potent, used in minute dosage form and are easily palatable and hence they are used in wide range in classical Ayurveda practice. Durjalajeta Rasa is an important herbo-mineral formulation mentioned in Rasa Chandamshu1. It contains a visha dravya and other subsidiary drugs. Utility of visha dravyas after their proper purification is also an integral part of Rasa shastra. It is quoted that if the vishes are administered in proper way, they give benefits of nectar. The main ingredients of Durjalajeta Rasa are Shuddha vatsanabha (Aconitum ferox Wall.), Kapardika Bhasma (Cypraea moneta Linn.) and Maricha (Piper nigrum Linn.). It is mainly indicated in vishama jwara, ajcarna, adhmsaan, shoolja, vishtamthba, kasa and shwasa. This formulation is prepared without giving agi samshkara and hence it is called as khalviyarasayana.

Durjalajeta Rasa is prepared in three batches to set the SOP. To standardize the sample the analytical testing is important for all three batches. Hence analytical study of final product is carried out and results are documented.

MATERIAL AND METHODS

All the ingredients for this formulation are procured from local authentic market and authenticated at the Quality Control laboratory of Muniyal Institute of Ayurveda Health Sciences, Manipal, Karnataka. All these herbal ingredients are passed quality parameters described in API.

Table 1: Pharmaceutical processing carried out during this study is as follows

| 1. Shodhana of Vatsanabha |
| 2. Preparation of Kulattha kwatha |
| 3. Shodhana of Kapardika |
| 4. Marana of Kapardika |
| 5. Preparation of powder of Shuddha Vatsanabha and Maricha |
| 6. Extraction of Ardaka swarasas |
| 7. Preparation of Durjalajeta Rasa pills |

Shodhana of Vatsanabha

150 g of Vatsanabha is subjected to nimajjan i.e. immersion in liquid. This procedure is done for three days by using fresh gomutra each time and kept under strong sunlight and resulting in 153 g of Shuddha Vatsanabha.2

Preparation of Kulattha kwatha

500 g of Kulattha (Horse gram) is added with 4 liters of water and boiled on moderate heat and reduced to ¼th i.e. resulting in 1 litre of kulatthakwatha.3

Shodhana of Kapardika

For shodhana process 300 g Kapardika is taken. Shodhana is carried out heating in Dolayantra using Kulatthakwatha as liquid
medium. Swedana is done for 3 hours on moderate heat and resulting in 260 g of Shuddha Kapardika.4

Marana of Kapardika

For the incineration process about 260 g of shuddha kapardika is taken. The shuddha kapardika is placed in a Sharava and done proper Sandhi bandhana (7 mud smeared layers). Then it is subjected to one Gaja Puta. After self-cooling it is collected and powdered for further Gajaputa. 240 g of powdered kapardika is triturated with kumara swarasa in khalwa yantra for 3 hours manually.5

Preparation of Shuddha Vatsanabha and Maricha powder

Shuddha Vatsanabha and Maricha is powered by pounding in khalwa yantra and later filtered with the help of sieve no. 120 to get fine powder.

Preparation of Durjalajeta Rasa pills

The pills are prepared with the reference of Rasa Chandamshu. All the ingredients are mixed homogeneously in khalwa yantra and triturated with the help of fresh andraka swarasa for 6 hours and pills are made of the size of 60 mg. Similarly, three batches are prepared.

Table 2: Ingredients of Durjalajeta Rasa with quantity

| Ingredients                        | Ratio   | Quantity |
|------------------------------------|---------|----------|
| Shuddha vatsanabha churna          | 2 parts | 6 g      |
| Kapardika bhasma                   | 5 parts | 15 g     |
| Maricha churna                     | 9 parts | 27 g     |
| Andraka swarasa – Bhavana dravya   | Q.S.    | 100 ml   |

Analytical study

To ensure reproducibility of Durjalajeta Rasa, the analytical methods are applied to three samples which are prepared with the same ingredients following the same standard manufacturing process and are coded as samples DJR1, DJR2 and DJR3. The final product is analyzed to obtain basic quality control parameters such as Average weight, Uniformity of weight of pills, Disintegration test, Hardness, Friability, Thin layer chromatography and subjected for advanced instrumental methods of analysis like X-Ray Diffraction (XRD), Scanning Electron Microscope-Energy Dispersive X-Ray Spectroscopy (SEM-EDAX), Inductively Coupled Plasma Mass spectroscopy (ICP-MS) and High performance Thin layer Chromatography (HPTLC).

1. Organoleptic parameters

(a) Colour
(b) Odour
(c) Taste
(d) Touch

2. Physico-chemical parameters

(a) Loss on drying
(b) Extractive values in water and alcohol
(c) Ash Value (total and acid insoluble ash)
(d) pH value6

3. Analysis of pills

a) Average weight
b) Uniformity of weight of pills
c) Hardness test
d) Disintegration time
e) Friability test

4. TLC and HPTLC6,9
5. Semi-Quantitative estimation of different elements by EDAX
6. Quantitative estimation of elements by ICP-MS10
7. X– Ray Diffraction11

Average weight

Randomly selected 10 pills are weighed and average weight is calculated.

Uniformity of weight of pills

A group of 10 pills is selected at random, weighed and calculated the average weight. Later the weight of individual pill is compared with the average weight of pills. In case of weight variation, it should fall within the permissible range of:

± 10% for pills weighing 120 mg or less
± 7.5% for pills weighing 120 mg to 300 mg
± 5% for pills weighing more than 300 mg

The test is said to be correct if not more than one in ten pills falls out of range.

Hardness test

The pills to be tested is held firmly held between two jaws and the scale is adjusted to 0 (zero). The knob is slowly and gradually turned so that the mobile jaw moves till it crushes the pill into powder. The reading in the tester is noted. The same procedure is repeated for 10 pills of the same weight.

Instrument – Monsanto Hardness Tester

Disintegration test

Apparatus used: Tablet disintegration apparatus.

The temperature is adjusted between 35-37°C. Around 500 ml of distilled water is added with 1 ml of concentrated HCl in the beaker. Introduce one pill into each tube and added a disc to each tube. The assembly is suspended in beaker containing the distilled water with HCl at specified temperature for specified time. The tube is allowed to move up and down for 30 times per minute. The pill passes the test if all of them have disintegrated. If one or two pills fail to disintegrate, repeat the test on additional 12 pills, not less than 16 of the totals of 18 pills tested disintegrate.

Friability test

Apparatus-Roche friabilator

10 number of whole pills is taken and those are dedusted carefully and each pill is weighed accurately. All the 10 pills are placed in a drum and the drum is rotated 100 times. Then the pills are removed, the loosed particles also removed carefully, and the pills are weighed accurately. During the procedure proper care should be taken that the pills should not be bound to each other.
Thin layered chromatography

Thin layer chromatography is a technique in which a solute undergoes distribution between two phases, stationary phase acting through adsorption and a mobile phase in the form of a liquid. By comparing the $R_f$ values of sample and reference substances, the component can be identified by its standard. Based on API standards for maricha, which is major ingredient of Durjalajeta Rasa. Toluene: Ethyl acetate (7:3) is used as mobile phase. Alcohol extract of products are spotted to develop chromatogram. Visualization is done under short wave UV light. (Table 11)

HPTLC

TLC Aluminum pre coated plate with silica gel 160 GF254 (20 × 10 cm²; 0.2 mm thick) is used with Toluene: Ethyl acetate: Hexane (6:3:1) V/V as mobile phase. Absolute alcoholic extract of samples and Piperine standard solution applied on plate by using Linomat V applicator. Cammag Twin Trough Glass Chamber (20 × 10 cm²) with SS lid is used for development of TLC plate. HPTLC plate is developed to 8 cm distance above the sample application. After the development, the plate is allowed to dry in air and examined under UV light rays 254 nm and 366 nm. In current study Piperine is used as the standard marker. Wincats an integrated Software 4.02 is used for detection. (Table 12)

SEM-EDAX

A Scanning Electron Microscopy may be equipped with an EDAX analysis system to enable it to perform compositional analysis on specimens. EDAX analysis is useful in identifying materials and contaminants, as well as estimating their relative concentrations on the surface of the specimen. Quantitative elemental composition is carried out by EDAX (based on ZAF Method Standard less Quantitative Analysis) at an acceleration voltage of 20 keV using the instrument 6380 (LA) at a voltage 20.0 kV, probe current of 1.0000 nA and PHA mode T4. An average mass percentage value of the elements from 3 samples of Durjalajeta Rasa is shown (Table 9).

ICP-MS

Inductively Coupled Plasma Mass Spectroscopy also referred as ICPMS is an analytical technique for detection of trace metals and elements. The sample is prepared in microwave digester by digesting the sample (0.5 g) with 3 ml concentrated HNO₃. (Table 10)

X-ray diffraction

X-ray diffraction has been using for the fingerprint characterization of crystalline materials and the determination of their structure. XRD patterns are obtained using a Shimadzu XRD-6000 diffractometer with Cu K-α as target with 40 kV voltages and 30 mA current. The crystallinity Durjalajeta Rasa is analyzed using an X-Ray diffractometer at NIO, Goa by irradiating with Cu – Kα radiation (at 1.54060Å). The analysis is performed from 20.0 to 80.0°(2θh) with a step size of 0.2°(2θh). Goniometer with the radius 240 mm is having a minimum step size of 0.001°(2θh) is used. The X-ray diffraction of the samples is matched against the standard reference spectra library of software for phase identification.

DISCUSSION

In the present study, Durjalajeta Rasa is prepared by adhering to Standard Operative Procedure. In this study three batches of trial drug are prepared by giving special importance starting from procurement of raw materials to finished product. Final product of Durjalajeta Rasa is in form of pills and hence initially basic quality control parameters of pills are applied, apart from organoleptic and physico-chemical analysis.

Pills are spherical, rough, gray coloured and hard in consistency. They exhibited typical odour of pepper and pungent taste of pepper. On evaluation Durjalajeta Rasa samples showed an average loss on drying 7.07% w/w which is within an acceptable limit. On an average total ash is 38.88% w/w due to addition of herbal ingredients. On an average 4.42% w/w is the acid insoluble ash which is quite low. Lower acid insoluble ash indicates better physiological availability. In all the three samples the quality control parameters do not show significant difference in their value.

All the samples of Durjalajeta Rasa are subjected for advanced instrumental methods of analysis. XRD pattern of all the three samples are much similar. All the 3 samples have shown the major peak with 100% intensity corresponding to that of calcite. When SEM images of Durjalajeta Rasa are taken there are scattered crystals of calcite which are hexagonal in shape. There are plenty of non-crystalline amorphous materials which may be due to organic substances coming from the herbal ingredients. In this work EDAX is carried out in association with SEM. Carbon and oxygen are found as major elements which are probably from the organic materials from herbal source. Calcium, Magnesium, Sulphur, Potassium and chloride are observed in all the samples.

Compared to AES, ICPMS have greater speed, precision and sensitivity. All the samples are tested quantitatively for the presence of elements like Calcium, Sodium and Potassium. On an average this element are present at a concentration of 5.36%, 0.076% and 0.512% respectively. Gomutra contains minerals like Chlorine, Magnesium, Calcium and Sulphur that are observed in final product. Kumari also contains minerals like Potassium, Phosphorus, Magnesium, Sodium and Zinc. Maricha is an integral component of Durjalajeta Rasa. Also contains minerals like Calcium, Magnesium, Zinc and Iron.

Based on API standards for maricha, which is major ingredient of Durjalajeta Rasa. Seven spots are visualized in maricha under 254 nm UV light which is used as reference drug. At least five compounds with $R_f$ values closely resembling to maricha are observed in 1st and 2nd sample of Durjalajeta Rasa whereas 3rd sample showed four compounds. A compound with $R_f$ value 0.36 seen in 1st sample and 0.38 seen in 3rd sample are most probably due to Vatsanabha.

HPTLC is very useful tool for standardization of poly herbal Ayurvedic formulations. In current study it is planned to use Piperine as the standard marker with $R_f$ value of 0.36 and to identify its presence as a qualitative marker in Durjalajeta samples. Toluene: Ethyl acetate: Hexane (6:3:1) is the mobile phase used. All the samples showed the presence of Piperine with an average value of 0.48% and it can be said that Piperine can be used as standard marker for the quality assurance of Durjalajeta Rasa by HPTLC method.
Table 3: Physical examinations of Vatsanabha before and after shodhana

| Tests                  | Ashudhha Vatsanabha | Shuddha Vatsanabha |
|------------------------|---------------------|--------------------|
| Consistency            | Hard and rough      | Becomes soft       |
| Colour                 | Dull brownish black | Pale yellow        |
| Touch                  | Hard, solid         | Soft               |
| Smell                  | No specific smell   | Gomutra smell      |

Table 4: Final quantity obtained of Kapardika bhasma after incineration

| Puta | Total weight of Kapardika before Puta | Total weight of Kapardika after Puta | Loss | % of loss |
|------|--------------------------------------|-------------------------------------|------|----------|
| 1st Puta | 300 g                               | 270 g                               | 30 g | 10%      |
| 2nd Puta | 250 g                               | 227 g                               | 23 g | 9.2%     |
| 3rd Puta | 202 g                               | 184 g                               | 18 g | 8.91%    |

Table 5: No. of pills obtained in each batch of Durjalajeta Rasa

| BATCH   | Total weight | Weight of each pills | Total no. of pills | Loss | Percentage of loss |
|---------|--------------|----------------------|--------------------|------|--------------------|
| DJR1    | 52 g         | 60 mg                | 698                | 10.08 g | 19.38%             |
| DJR2    | 55 g         | 60 mg                | 705                | 10.68 g | 20.15%             |
| DJR3    | 52 g         | 60 mg                | 695                | 10.26 g | 19.73%             |

Table 6: Organoleptic analysis Durjalajeta Rasa

| Parameters | DJR 1 | DJR 2 | DJR 3 |
|------------|-------|-------|-------|
| 1. Colour  | Gray  | Gray  | Gray  |
| 2. Odour   | Peppery smell | Peppery smell | Peppery smell |
| 3. Taste   | Pepper taste | Pepper taste | Pepper taste |
| 4. Touch   | Hard rough | Hard rough | Hard rough |

Table 7: Physico-chemical parameters of Durjalajeta Rasa

| S. No. | Parameters                  | DJR 1          | DJR 2          | DJR 3          |
|--------|----------------------------|----------------|----------------|----------------|
| 1      | LOD (Loss on drying)       | 7.58 % w/w     | 5.60% w/w      | 8.04% w/w      |
| 2      | Total ash                  | 32.7% w/w      | 34.55% w/w     | 31.40% w/w     |
| 3      | Acid insoluble ash         | 6.4% w/w       | 3.66% w/w      | 3.2% w/w       |
| 4      | pH                         | 9.67           | 9.89           | 9.76           |
| 5      | Extractive value in water  | 19.54% w/w     | 12.89% w/w     | 10.72% w/w     |
| 6      | Extractive values in alcohol | 11.72% w/w   | 7% w/w         | 6.69% w/w      |

Table 8: Analysis of pills

| S. No. | Test                  | DJR1          | DJR2          | DJR3          |
|--------|-----------------------|---------------|---------------|---------------|
| 1      | Average weight        | 61 mg         | 62 mg         | 64 mg         |
| 2      | Uniformity of weight of pills | -8.19 to + 6.55 | -9.67 to + 9.67 | -7.81 to + 6.25 |
| 3      | Hardness              | 1.75          | 1.65          | 1.75          |
| 4      | Disintegration time   | 7 mins        | 6 mins        | 6 mins        |
| 5      | Friability            | 0.14%         | 0.30%         | 0.15%         |
| 6      | TLC                   | Complies      | Complies      | Complies      |

Instrumental methods of analysis

Figure 1: XRD graph of DJR1
Table 9: Average values of the elements from 3 samples of Durjalajeta Rasa

| Element | Mass % of DJR |
|---------|--------------|
| C       | 25.71        |
| O       | 70.10        |
| Mg      | 0.10         |
| S       | 0.10         |
| Cl      | 0.12         |
| K       | 0.38         |
| Ca      | 3.48         |

Table 10: Elemental composition in Durjalajeta Rasa samples by ICP-MS

| S. No. | Tests | DJR1          | DJR2          | DJR3          |
|--------|-------|---------------|---------------|---------------|
| 1      | Ca    | 5.27%         | 5.65%         | 5.16%         |
| 2      | Na    | 0.0846%       | 0.08%         | 0.0620%       |
| 3      | K     | 0.52%         | 0.6102%       | 0.404%        |

Table 11: TLC findings of Maricha and alcoholic extract of Durjalajeta Rasa samples

| Sample name | Wavelength | No. of spots | Rf value | Colour of spot |
|-------------|------------|--------------|----------|----------------|
| Maricha     | 254 nm     | 6            | 0.55     | Light brown    |
|             |            |              | 0.65     | Light brown    |
|             |            |              | 0.72     | Dark brown     |
|             |            |              | 0.73     | Light yellow   |
|             |            |              | 0.80     | Light yellow   |
|             |            |              | 0.88     | Dark brown     |
| DJR1        | 254 nm     | 5            | 0.36     | Light yellow   |
|             |            |              | 0.55     | Light brown    |
|             |            |              | 0.62     | Dark brown     |
|             |            |              | 0.76     | Light yellow   |
|             |            |              | 0.83     | Light brown    |
| DJR2        | 254 nm     | 5            | 0.52     | Light brown    |
|             |            |              | 0.62     | Dark brown     |
|             |            |              | 0.69     | Light yellow   |
|             |            |              | 0.76     | Light yellow   |
|             |            |              | 0.83     | Light brown    |
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| Sample ID | Piperine content |
|-----------|------------------|
| DJR1      | 0.54%            |
| DJR2      | 0.48%            |
| DJR3      | 0.43%            |

Table 12: Piperine content in three samples of Durjalajeta Rasa

HPTLC profile

Figure 4: HPTLC Fingerprints of Durjalajeta Rasa Pills

Figure 5: Shuddha Vatsanabha

Figure 6: Maricha fruit

Figure 7: Kapardika Bhasma

Figure 8: Ardraka swarasa

Figure 9: Bhavana process

Figure 10: Durjalajeta Rasa Pills
CONCLUSION

From the above study we can conclude that the Durjalajeta Rasa (pills) formulated by the method explained in the Rasa Chandamshu in three batches do not show any significant difference in the physicochemical and in advanced instrumental analysis. Hence, we can say that Durjalajeta Rasa (pills) prepared by this method complies the standards parameters. Therefore, the mentioned pharmaceutical and analytical parameters for Durjalajeta Rasa (pills) are valid and standard one.

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