Research Article

PHARMACEUTICAL AND ANALYTICAL STUDY OF TRINETRA RAS – HERBO-MINERAL FORMULATION

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ABSTRACT

Trinetras is a Kharliya Rasayan mentioned in Yogratnakar for the treatment of Hridroga (Yogratnakar Uttarardha Hridroga Chikitsa 1,2). It consists of Shuddha Parad, Shudhagandhak and Abhrakbhasma where Bhavanadravya is Arjuna bark decoction. **Aim:** Pharmaceutical and analytical study of Trinetras. **Materials and methods:** Paradshodhan, Gandhakshodhan, Vajrabhrakshodhan, Dhanyabhrraknirmana, preparation of Abhrakbhasma, preparation of Kajjali are the procedures required to be performed before the preparation of Trinetras. Total 40 times Abhrak was subjected to Maransanskar in Gajaputa (40 puti Abhrakbhasma). Trinetras can be prepared from Shuddha Parad, Shudhagandhak and Abhrakbhasma, Arjuna bark decoction is needed for 21 Bhavana. Total three batches of Trinetra Ras were prepared as per the classical method mentioned in the reference. **Observations and results:** Prepared samples of Trinetras and Abhrakbhasma were tested on the basis of organoleptic and physicochemical parameters. Along with Ayurvedic parameters modern parameters such as L.O.D., L.O.I., pH, conductivity, successive solubility, elemental assay of Hg, S and Fe, TLC, phenolic assay etc. were applied to the samples of Trinetras. **Conclusion:** Analytical study of Trinetras revealed the uniformity of the procedures in the three samples of Trinetras, as evidenced by the observations of the analytical values of the three samples were not much variation found. Analytical profile showed the presence of the ingredients used. Data generated from pharmaceutical, analytical studies can be used to develop a preliminary standard profile for the formulation of Trinetra Ras.

**KEYWORDS:** Pharmaceutical, Analytical, Trinetra Ras, Parad, Gandhak, Shodhan, Abhrak Bhasma.

INTRODUCTION

The word Rasashastra literally means the science of Mercury. It is a specialized branch of Ayurveda dealing mainly with materials which are known as Rasadravyas. It mainly revolves around Mercury and its preparations. The products dealt under this discipline are an important component of Ayurvedic therapeutics.

In the present renaissance of Ayurveda, it is the need of time to have a pharmaceutical and analytical study to re-establish, represent and to update techniques of preparation of Ayurvedic medicine.

Considering the principals of Ras-shastra and Bhaishajya Kalpana and also the importance of their standard method of preparation, it is decided to carry out pharmaceutical and analytical study of Trinettras.

Trinetras is mentioned in the Yogratnakar for therapeutic use in Hridroga.

Identity of Hridroga was established from the classical age of Ayurveda as its description and management is available in all classics of Ayurveda. In current practice Ayurveda has fewer roles in cardiac emergencies but definitely there is supreme role in cardio protective activity.

There are several formulations available in the text of Ayurveda for the treatment of Hridroga. Trinetras is one of them. Trinetras is a Kharliya Rasayan mentioned in Yogratnakar for the treatment of Hridroga [Yogratnakar Uttarardha Hridroga Chikitsa (1,2)]. It consists of Shuddha Parad, Shuddha gandhaka and Abhrakbhasma where Bhavanadravya (material for trituration) is Arjunatwakkwath (decoction of stem bark of Terminalia arjuna).

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The present study Pharmaceutical and analytical study of Trinetraras was undertaken to understand and prepare them according to text and analyze them in terms of Ayurvedic and modern parameters to know their significance.

AIMS AND OBJECTIVES

The aim of the present work was to study the Pharmaceutical and Analytical Study of Trinetraras.  
1) Preparation of Trinetraras as per scientific concepts mentioned in Classical Texts of Rasshastra.
2) Study of Prepared drug for Organoleptic and Physico-chemical parameters.

MATERIALS AND METHODS

In this topic following studies are included.

Pharmaceutical Study

In this study right from the collection of raw material, their Shodhan, Maran, and the preparation of Trineta Ras was done and procedure was repeated thrice.

Analytical Study

In this study Ayurvedic and modern parameter regarding to Trinetraras are studied.

Pharmaceutical Study

The following processes regarding to Trinetraras are studied.

Collection of Raw material

Raw materials were collected from authorized market according to their description mentioned in the texts, further authentified by the experts.

Parad Shodhan\(^1\)

Equipments

Weighing machine, Khalvayantra, steel vessel, earthen vessels, gas, cloth for filtration of decoction.

Ingredients

\[
\begin{align*}
\text{Ashuddha Parad} & : 500 \text{ gm} \\
\text{Kumariswaras} & : 100 \text{ mL} \\
\text{Chitrakamoolo} & : 100 \text{ gm} \\
\text{Raktasarshapa} & : 100 \text{ gm} \\
\text{Brihati} & : 100 \text{ gm} \\
\text{Triphala} & : 100 \text{ gm} \\
\text{Water for Kwath} & : 3.2 \text{ lit} \\
\end{align*}
\]

Total Kwath prepared \(-0.8 \text{ lit}\)

Procedure

Chitrakamoolo, Raktasarshapa, Brihati and Triphala was taken in steel vessel each 100 gm, eight times water added, kept on gas reducing it to one fourth. 500 gm of Ashuddha Parad was taken in Khalvayantra. Then freshly prepared Kwath and Kumariswaras added in it. This mixture was triturated well together. On second day trituration was started using freshly prepared Kwath and Kumariswaras. Same procedure was repeated for 6 days; approximately 5-6 hrs daily trituration was done till Parad fully disintegrated into fine particles. At the end of sixth day Parad was completely disintegrated into fine powder, and then it was allowed to stand still overnight. On seventh day Kwath from Khalvaayntra was separated slowly without disturbing settled Parad. Removed Kwath was allowed to stand and settled Parad was collected. It was washed with lime juice and lukewarm water then filtered. Shuddha Parad was dried completely and stored in airtight glass bottle. Same procedure was repeated for another 2 samples.

Duration: 28-30 hours (daily 4-5 hrs) for each sample. Total duration is 22 days.

Gandhaka Shodhana\(^2\)

Equipments

Khalvaayntra, Weighing machine, gas stove, Iron pan, cloth for filtration, Steel jar, thread etc.

Ingredients:

\[
\begin{align*}
\text{1) Ashuddha Gandhaka} & : 500 \text{ gm} \\
\text{2) Goghrita} & : 500 \text{ gm} \\
\text{3) Godugdha} & : 2 \text{ lit} \\
\end{align*}
\]

Procedure

Goghrita (cow-ghee) was taken in a clean iron pan, heated it on Mandagni and powder of Gandhaka was added to it. The mixture was stirred continuously. On another side a stainless steel container filled with Godugdha was taken and cloth tied at the mouth of vessel. When the Gandhaka liquefied completely, heating was stopped and poured on the cloth to fall in Godugdha. After cooling, solid slab of Gandhaka was taken out and washed with hot water till the adhered Goghrita removed completely. Same procedure was repeated 2 times. The pure Gandhak obtained was dried, powdered and stored. This procedure was repeated for another 2 samples.

Duration: 2 days for each sample (total 6-7)

Vajrabhrakashodhan\(^3\)

Equipments- Steel vessel, Bhatti, metal tongs, cloth, measuring jar, weighing machine, iron pan etc.

Ingredients

\[
\begin{align*}
\text{Ashuddha Abdhraka} & : 500 \text{ gm} \\
\text{Triphala Kvatha} & : 1000 \text{ mL} \\
\end{align*}
\]

Procedure

Required amount of Triphala Kvatha was taken in a steel vessel. Raw Abdhraka chips were kept in Bhatti till they became red hot. The Abdhraka chips were turned up and down with metal tongs, when the Abdhraka chips became completely red hot, they were quenched (Nirvapana) into the Triphala Kvatha. After

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few minutes the Triphala Kwatha was separated and pieces of Abhraka were collected in an iron pan to subject it for next Nirvapana. Same procedure was repeated for 7 times. The entire procedure was repeated for another two samples.

**Duration:** 3 days

**Dhanyabhraka Nirmana**

Equipment- Jute cloth, jute yarn, steel vessels, tray, spatula, plastic pot etc.

Ingredients

| Shuddha Abhraka | 465 gm |
| Dhana | 120 gm |
| Water | q.s. |

**Procedure**

Firstly Shuddha Abhraka was transferred to a tray and ¼ quantity of Dhana with respect to Abhraka was added layer by layer. Then a jute cloth was spread on a table and mixture of Dhana and Abhraka was transferred on it. After covering it, a Pottali was tied by a jute yarn. Then required amount of water was taken in a plastic pot and the Pottali was dipped in it completely for 72 hours. More water was added when the quantity of water was reduced, so as to keep Pottali completely dipped in it. On the 4th day, Pottali was taken out. A large plastic vessel containing water was taken. A Pottali was dipped in it and rubbed. When the colour of water changed to black, a new pot with water was taken to give fresh media. The process is continued till the extraction of Dhanyabhraka. The upper clean water in the pot was separated after sedimentation and the residue was allowed to soak. At last lustrous black coloured fine powder of Dhanyabhraka was collected.

**Duration:** 7 days

**Preparation of Abhrakbhasma**

**Equipments:** Weighing machine, spatula, Mruttikasharava, cloths for Sandhibandhan

**Ingredients**

| Dhanyabhrik | 200 gm |
| Jaggary | 200 gm |
| Erandapatraswaras | q.s. |

**Procedure**

Dhanyabhrik and Guda taken in Khalvayantra and triturated well together. Erandapatraswaras was added in it and triturated for 3 hours. Similar sized small circular cakes (Chakrika) were made. Chakrika were kept in Sharava and another Sharava was covered over it, Sandhibandhan was done in three layers. This Sharvasamputa were shed dried and subjected for Maranasanskara. In cuboidal pit cow dung cakes were filled 2/3 then Sharvasamputa kept and remaining 1/3 covered with cow dung cakes. After cooling down the Sharava were collected and observations noted. This procedure was repeated for 40 times. Same procedure was done for all 3 batches.

**Puta Upala (Cow dung cakes)**

Cow dung cakes were collected from the same place to avoid variations about size and weight.

Calculation of cow dung: Average weight of cow dung = 220 gm

**Preparation of Kajjali**

**Equipments:** Weighing machine, Khalvayantra, spatula, steel plate etc.

**Ingredients**

| Shuddha Parada | 200 gm |
| Shuddha Gandhaka | 200 gm |

**Procedure**

Shuddha Parada was taken in a Khalvayantra; equal amount of Shuddha Gandhaka was added to it and triturated. Gradually the white colour of Parada and yellow colour of Gandhaka disappeared and a black powder was formed. Trituration (Mardana) was continued till the powder became black in colour, very fine in consistency, Varitar, Shlakshna and Nishchandra. Same procedure was repeated for another two samples.

**Duration:** 5-6 days (6 hours per day) for each sample, total-18 days

**Preparation of Trinetra rasa**

**Reference:** Yogaratnakar Hirdaroga Chikitsa (1/2)

**Name of the procedure:** Bhavanasanskar (Trituration)

**Equipments:** weighing machine, measuring jars, Khalvayantra

**Ingredients**

| Shuddha Parad | 50 gm |
| Shuddha Gandhak | 50 gm |
| Abhrakbhasma | 50 gm |
| Arjuna bark decoction | 200 mL |

**Procedure**

Kajjali was prepared by taking equal amount of Shuddha Parad and Gandhak. Abhrakbhasma was added to above mixture. This mixture was triturated with Arjuna bark decoction. This procedure was repeated for 21 times. The same procedure was repeated for another 2 samples.

**Duration:** 12 days

**Precautions**

During each time freshly prepared decoction was used. Before each Bhavana mixture was completely dried.
Analytical Study

Ayurvedic Parameters for Abhraka Bhasma

Nishchandra

Nishchandra is the specific parameter for Abhraka Bhasma. A portion of prepared Abhrakabhasma was rubbed in between fingers and thumb and the rubbed portion was examined in sun’s rays. This test was performed with the naked eye and microscopically.

The prepared samples of Abhrakabhasma were found to be Nishchandra.

Varnotapatti

It indicates the colour of the Bhasma. A specific colour is mentioned for each Bhasma and alternation in this specific colour suggests that the Bhasma is not prepared properly. Because a particular compound is formed during Bhasma preparation and every chemical compound possess specific colour.

As per Rasaratnasamuchaya Abhrakabhasma should be Sindurabha coloured. All the prepared 3 samples of Abhrakabhasma were Sindurabha coloured.

Sooksha (Anjana-sadrusha)

The Bhasma on application to eyes as Kajal, which does not cause any irritation proves the maximum fineness and softness of Bhasma.

Rekhapurnatva

This test indicates the fineness of a Bhasma. The Bhasma was rubbed in between the thumb and index finger. The particles of the Bhasma attained such a state that the Bhasma could settle in the ridges of the fingers.

All the 3 samples of Abhrakabhasma possessed this character.

Sparshakomal/ Mrudutva and Shlakshnata

The softness and smoothness of the Bhasma is also due to its fineness. Touching Bhasma by fingers and feeling of touch was noted.

All the 3 samples of Abhrakabhasma were found Mrudu and Shlakshna i.e., Sparshakomal.

Nirdhoom

When there is any moisture or organic content or sulfur present in Bhasma, fumes are produced on its burning.

Hence this test of Bhasma was performed by taking it in small quantity in silica crucible and ignited. The ignition of Bhasma was observed carefully and observations noted. Fumes did not produce hence Bhasma found Nirdhoom.

Niswadu

The properly prepared Bhasma attains tastelessness. The presence of taste in Bhasma indicates the imperfectness of Bhasma.

All the three prepared 3 samples of Abhrakabhasma were found tasteless (Niswadu).

1. Physico-Chemical Parameters
2. Loss on Drying
3. Loss on Ignition (L.O.I.)
4. Determination of Conductivity
5. Determination of Ph
6. Successive Solubility

Successive solubility of Trinetraras was carried out in CS₂, H₂O, Dilute HCL, Moderate concentrated HNO₃ and Aquaregia.

Successive solubility of Abhrakabhasma was carried out in H₂O, Dilute HCL, Moderate concentrated HNO₃ and Aquaregia.

Elemental Assay

1. Estimation of Mercury
2. Estimation of Sulfur
3. Estimation of Total iron in Trinetraras
4. Estimation of Ferrous ions in Abhrakabhasma
5. Estimation of Ferric ions in Abhrakabhasma

Phenolic Assay

Phenols includes an array of compounds like tannins, Flavonols etc. Total phenol estimation can be carried out with the Folin-Giocealteau reagent.

Thin Layer Chromatography

It was run on silica gel G-254 F (Merck)in solution Toulene: Ethyl acetate: Formic acid = 7:3:0.5.

500 mg of Trinetra rasa and the Arjuna bark i.e. powdered Arjuna was soaked in 3mL of Ethyl alcohol for 24 hours. The filtrate was concentrated by evaporating in the water bath. The Ethanolic extract was applied on TLC plate in the form of band and run as follows.

OBSERVATIONS AND RESULTS

Pharmaceutical Study

Parad Shodhan

After 4 hours of trituration Parad started to disintegrate slowly and colour of the Kwath turned black. As trituration continued Parad disintegrated successively into fine particles. At the end of 12 hours small particles were formed. Some particles were so fine that floated on the surface of decoction and whitish layer was formed on the wall of mortar. Trituration was done till Parad appears like powdered form silvery grayish colour. While rinsing the Parad with luke warm water fine particles of Parad merged together as whole. The water after rinsing was allowed to stand to collect remaining Parad particles. After Shodhana, colour of Parad was silvery and brighter in appearance.
### Results

|          | P-1     | P-2     | P-3     |
|----------|---------|---------|---------|
| Initial weight | 500 gm  | 500 gm  | 500 gm  |
| Final weight   | 480 gm  | 476 gm  | 482 gm  |
| Weight loss    | 20gm    | 24 gm   | 18 gm   |

**Gandhaka Shodhan**

After melting Gandhaka turned into yellowish red colour. Strong pungent sulfur fumes came during melting. The physical impurities were trapped while filtering melted Gandhaka through the cloth. After pouring Gandhaka the blackish coloured Ghruta was floated on the surface of milk. Gandhaka was washed with hot water, till the Ghrutagandha was expelled completely. After Shodhan the Gandhaka acquires a granular yellow colour.

### Results

|          | G-1     | G-2     | G-3     |
|----------|---------|---------|---------|
| Initial weight | 500gm   | 500gm   | 500gm   |
| Final weight   | 474gm   | 470gm   | 468gm   |
| Weight loss    | 26gm    | 30gm    | 32gm    |

**Vajrabhrakashodhan**

After quenching of Vajrabhraka into Triphalakvath colour of the media was changed from brown to blackish brown. At the end of procedure large sized Vajrabhraka get converted into smaller sized pieces of Vajrabhraka. Shodhita bhrika was brittle in nature with decreased lusture.

### Results

|          | A-1     | A-2     | A-3     |
|----------|---------|---------|---------|
| Initial weight | 465 gm  | 460 gm  | 456 gm  |
| Final weight   | 410 gm  | 410 gm  | 415 gm  |
| Weight loss    | 55 gm   | 50 gm   | 41 gm   |

### Preparation of Dhanyabhraka

The prepared Dhanyabhraka was uniformly fine powdered form with decreased lusture. The particles of the Dhanyabhraka were so fine that it moves with water and were suspended in the water. Silica and stones along with Dhana were trapped in the Pottali.

### Results

|          | A-1     | A-2     | A-3     |
|----------|---------|---------|---------|
| Initial weight | 465 gm  | 460 gm  | 456 gm  |
| Final weight   | 410 gm  | 410 gm  | 415 gm  |
| Weight loss    | 55 gm   | 50 gm   | 41 gm   |

### Preparation of Abhrakabhasma

In the first 5 Gajaputa Abhrakbhasma was black coloured, hard in consistency and there was increase in weight of Abhrakbhasma. At the end of 11 Puta Abhrakbhasma was black with greyish shade, soft in consistency, complete Rekhapurnatva appeared in the Bhasma. From Puta no.12 to17 Abhrakbhasma was gray in colour and soft in consistency. From Puta no.18 to 23 Abhrakbhasma was brick red in colour, soft in consistency with successive decrease in Chandrika. At the end of 24th Gajaputa desired colour Sindurabha appeared in the Abhrakbhasma with decrease in Chandrika. From 25th Puta to 34th Putas Indhurabha colour present in Abhrakbhasma with complete loss of Chandrika in successive Puta. At the end of 34th Puta Abhrakbhasma possessed all the characteristics Ayurvedic parameters. Further Puta no.35 to 40 were given for therapeutic potentiation of Abhrakbhasma.

**Graph 1: Showing observations regarding temperature during Maransanskar of Abhrakabhasma**
Preparation of Kajjali

Initially Parad was moving freely in Kharal while triturating. After 1 hour, the colour of the mixture started changing from yellowish to grey. After 2 hours, 50% of Parad disappeared. After 6 Hours whole mixture turned into blackish powder but a few shining particles of Parad were observed. After 10 hours 50% of shining particles disappeared. After 15 Hours Rekhapurnatva appeared in the Kajjali. After 18 hours Varitaratva appeared in the Kajjali.

Preparation of Trinetraras

Showing observations of preparation of Trinetraras

Analytical Study

Ayurvedic Parameters for Bhasma pariksha revealed the results as follows

Prepared samples of Abhrakabhasma (AB-1, AB-2, AB-3) were Nischandra, Sindurabha coloured, Sookshma, Rekhapurna, Sparshakomal (Mrudu, Shlakshna), Nirdhum and Niswadu.

Table 1: Showing Organoleptic characters of Abhraka Bhasma

| S.No. | Samples | Colour (Rupa) | Odour (Gandha) | Taste (Rasa) | Sound (Shabda) | Sparsha (Touch) |
|------|---------|--------------|----------------|--------------|---------------|----------------|
| 1    | AB-1    | Sindurabha   | Odourless      | Tasteless    | (Niswadu)     | -              |
|      |         |              |                |              |               | Soft smooth powdered form |
| 2    | AB-2    | Sindurabha   | Odourless      | Tasteless    | (Niswadu)     | -              |
|      |         |              |                |              |               | Soft smooth powdered form |
| 3    | AB-3    | Sindurabha   | Odourless      | Tasteless    | (Niswadu)     | -              |
|      |         |              |                |              |               | Soft smooth powdered form |

Table 2: Showing Organoleptic characters of Trinetraras

| S.No | Samples | Colour (Rupa) | Odour (Gandha) | Touch (Sparsha) | Sound (Shabda) | Taste (Rasa) |
|------|---------|--------------|----------------|-----------------|---------------|--------------|
| 1    | T.R-1   | Black        | Resembling Gandhak | Soft smooth powdered form | - | Madhur, Kashaya |
| 2    | T.R-2   | Black        | Resembling Gandhak | Soft smooth powdered form | - | Madhur, Kashaya |
| 3    | T.R-3   | Black        | Resembling Gandhak | Soft smooth powdered form | - | Madhur, Kashaya |

Physico-chemical parameters

Loss on drying

Table 3: Showing L.O.D.% of all six samples

| Sr. No | Samples | L.O.D. (%) |
|--------|---------|------------|
| 1      | AB-1    | 0.48       |
| 2      | AB-2    | 0.27       |
| 3      | AB-3    | 0.053      |
| 4      | T.R-1   | 1.89       |
| 5      | T.R-2   | 1.09       |
| 6      | T.R-3   | 2.82       |
Loss on Ignition

Table 4: Showing L.O.I. % of all three samples of Abhraka Bhasma

| Sr. No. | Samples | L.O.I. % |
|---------|---------|----------|
| 1       | AB-1    | 0.2      |
| 2       | AB-2    | 0.38     |
| 3       | AB-3    | 0        |

Table 5: Showing L.O.I.% of all three samples of Trinetraras

| Sr.No. | Samples | L.O.I.% |
|--------|---------|---------|
| 1      | T.R.-1  | 64.52%  |
| 2      | T.R.-2  | 69.08%  |
| 3      | T.R.-3  | 69.22%  |

Conductivity

Table 6: Showing Conductivity of all six samples

| Sr. No | Samples | Conductivity |
|--------|---------|--------------|
|        |         | 0. (µ mhos)  | 1% (µ mhos) |
| 1      | AB-1    | 150          | 990         |
| 2      | AB-2    | 135          | 780         |
| 3      | AB-3    | 126          | 840         |
| 4      | T.R.-1  | 150          | 660         |
| 5      | T.R.-2  | 132          | 600         |
| 6      | T.R.-3  | 111          | 600         |

pH Values

Table 7: Showing pH of all six Samples

| Sr.No. | Samples | pH of samples |
|--------|---------|---------------|
|        |         | 0.1% | 1% |
| 1      | AB-1    | 7.90 | 7.72 |
| 2      | AB-2    | 8.15 | 7.32 |
| 3      | AB-3    | 8.02 | 7.58 |
| 4      | T.R.-1  | 5.72 | 6.13 |
| 5      | T.R.-2  | 6.06 | 6.08 |
| 6      | T.R.-3  | 5.94 | 5.96 |

Successive solubility

Table 8: Showing percentage of Successive Solubility of Abhraka Bhasma

| S.No. | Samples | In H2O (%) | In Dil.HCl (%) | In Mod. Conc.HNO3 (%) | In Aquaregia (%) | Total % of Solubility |
|-------|---------|------------|----------------|-----------------------|------------------|-----------------------|
| 1     | AB-1    | 21.16      | 41.56          | 0                     | 0                | 62.72                 |
| 2     | AB-2    | 9.12       | 55.36          | 0                     | 0                | 64.48                 |
| 3     | AB-3    | 9.56       | 55.08          | 0                     | 0                | 64.64                 |

Table 9: Showing percentage of successive Solubility of Trinetra rasa

| S. No. | Samples | In CS2 (%) | In H2O (%) | In Dil.HCl (%) | In Mod. Conc.HNO3 (%) | In Aquaregia (%) | Total (%) |
|--------|---------|------------|------------|----------------|-----------------------|------------------|------------|
| 1      | T.R.-1  | 28.04      | 8.64       | 14.64          | 6.28                  | 29.52            | 87.12      |
| 2      | T.R.-2  | 26.6       | 4.88       | 17.84          | 7.6                   | 27.32            | 84.24      |
| 3      | T.R.-3  | 27.16      | 3.72       | 18.6           | 8                     | 29.2             | 86.68      |
Estimation of Elements

Estimation of Mercury

Table 10: Showing percentage of Mercury in all samples of *Trinetraras*

| Sr. No. | Samples | Mercury (Hg%) |
|---------|---------|---------------|
| 1       | T.R.-1  | 28.05         |
| 2       | T.R.-2  | 31.72         |
| 3       | T.R.-3  | 30.39         |

Estimation of Sulfur

Table 11: Showing percentage of Sulfur in all samples of *Trinetra rasa*

| Sr. No. | Samples | Total Sulfur (%) |
|---------|---------|------------------|
| 1       | T.R.-1  | 31.51            |
| 2       | T.R.-2  | 30.62            |
| 3       | T.R.-3  | 29.38            |

Estimation of total iron

Table 12: Showing Total Iron percentage in all three samples of *Trinetraras*

| Sr. No. | Samples | Total Fe% |
|---------|---------|-----------|
| 1       | T.R.-1  | 1.5635%   |
| 2       | T.R.-2  | 1.9655%   |
| 3       | T.R.-3  | 1.9655%   |

Estimation of Fe$^{2+}$ and Fe$^{3+}$ ions in *Abhrakabhasma*

Table 13: Showing percentage of Fe$^{2+}$ and Fe$^{3+}$ ions in *Abhraka Bhasma*

| Sr. No. | Samples | Fe$^{2+}$ % | Fe$^{3+}$ % |
|---------|---------|-------------|-------------|
| 1       | AB-1    | 1.2119      | 4.78        |
| 2       | AB-2    | 1.016       | 5.45        |
| 3       | AB-3    | 1.016       | 5.45        |

Phenolic assay

Table 14: Showing mg of phenolics per 100 gm in all three samples of *Trinetraras*

| Sr. No. | Samples | mg of Phenolics per 100 gm of T.R. |
|---------|---------|-----------------------------------|
| 1       | T.R.-1  | 209.07                            |
| 2       | T.R.-2  | 190.24                            |
| 3       | T.R.-3  | 204                                |

Thin layer chromatography

**TLC of *Arjuna***

*Trinetraras* – *T$_1$*

*Arjuna* – *T$_2$*

Plate – Silica gel G-254 F (Merck)

Solvent system – Tolune: Ethyl acetate:Formic acid =7:3:0.5

Solvent front =7cm

No spots were seen in visible light and UV light.

Table 15: Showing TLC bands with R$_f$ value

| Sr.No | R$_f$ value | With Iodine T1T2 | With Anisaldehyde T1T2 |
|-------|-------------|------------------|------------------------|
| 1     | 0.04285     | -                | + (Brown)              |
| 2     | 0.05714     | + (Brown)        | -                      |
| 3     | 0.1285      | + (Brown)        | -                      |
|       |             |                  | + (Blue) + (Blue)      |
| 4     | 0.1928      | + (Brown)        | -                      |
| 5     | 0.2357      | + (Brown)        | -                      |
| 6     | 0.2785      | + (Brown)        | -                      |
Abhrakabhasma

Abhrakabhasma was carried out according to reference of R.T.5/31 Observations were noted before and after Shodhan process. With the help of media used elimination of Parad dosha is achieved. The Shodhit Parad typically resembled as mentioned in the literature. Gandhakshodhan was done as per the reference of R.R.S.3/20-22 Shodhit Gandhak was yellow and brittle in nature. Physical impurities were trapped in cloth tied to the mouth of vessel and blackish coloured layer of Ghṛtṛa was floated on the surface of milk which clarifies the significance of Gandhan shodhan.

Preparation of Abhrakabhasma was carried out as per the reference of R.R.S.2/6-27. Jagary was subjected to Maransanskar in Gajaputa. At the end Sindurabhavarn was obtained. Puta wise observations were noted and procedure was repeated till it fulfills the Bhasma siddhi pariksha Nishchandratva, Sindurabha, Sookshma, Sparshakomal. Nishchandrapariksha was performed with the naked eyes and microscopically.

Kajjali was prepared as per the reference of R.T.6/107. Trituration of Kajjali was done till the parameters of Kajjalirekhapurnatva, varitaratva, Nishchandratva were fulfilled.

The preparation of Trinetraras was carried out as per the reference of Y.R.Hridrogi Chikitsa (1, 2). In this procedure Shuddha Parad and Shuddhagandhak were taken in equal proportion and Kajjali was prepared. To the above prepared Kajjali Abhrakabhasma was added in equal proportion to that of Parad and Gandhak (Shuddha Parad: Shuddhagandhak: Abhrakabhasma =1:1:1)

Above mixture was triturated well together with Arjuna bark decoction for 21 times.

Trinetraras and Abhrakabhasma were tested on the basis of organoleptic and physicochemical parameters. The prepared samples of Abhraka bhasma found to possess following characters; Nishchandratva, Sindurabhavarn, Rekhaapurnatva, Sookshma, Sparshakomal (Mrudutva/Shilikshnatva), Nirdhuma, Niswadu.

Estimation of L.O.D. of samples of Trinetraras stated that it has least moisture content. The drug is having least hygroscopic activity with less chances of contamination of drug. L.O.I. of Abhrakabhasma showed negligible loss as it is end product of successive Gajaputa. L.O.I. of samples of Trinetraras signifies volatile nature of Hg, S and organic constituents in it on ignition. Abhrakabhasma is slightly alkaline in nature whereas Trinetraras is slightly acidic in nature. Determination of conductivity of samples of Trinetraras determines the rich concentration of charged ions in the solution. Samples of Abhrakabhasma were maximum soluble in dil.HCl and insoluble in mod.conc. HNO3 and aqua regia whereas total solubility of the samples of Trinetraras T.R.-1,T.R.-2,T.R.-3 were 87.12%, 84.24%, 86.68%. Estimation of elemental assay Hg, S, Fe clearly signifies its presence and proportion in the prepared samples of Trinetraras. Prepared samples of Abhrakabhasma contains iron in ferrous (Fe2+) and ferric (Fe3+) forms providing better bioavailability. Phenols are pharmacologically active compounds present in Trinetraras are derived from Arjuna bark decoction.

CONCLUSION

Trinetraras can be prepared from Shuddha Parad, Shuddhagandhak and 40 Puti Abhrakabhasma with 21 Bhavana with Arjunatwakqwath. Abhrak bhasma subjected to more Gajaputa more will be its therapeutic efficacy. Hence future research is expected to elaborate its therapeutic utility in management of cardiovascular diseases.

Shodhan has broader concept other than purification. Shodhan is essential for the further pharmaceutical processes. Shodhan of Parad and
Gandhak typically resembles as stated in the reference. Preparation of Dhany Abhraka states the significance of concept used by ancient Acharya. Jaggary and Erandapatraswarasa are the best and suitable media for the preparation of Abhraka bhasma. Properly prepared Bhasma possess all the parameters described in Ayurvedic classics.

Analytical study of Trinetraras revealed the uniformity of the procedures in the three samples of Trinetraras, as evidenced by the observations of the analytical values of the three samples were not much variations found. Therefore physicochemical parameters serve as a mean for Standardisation of herbo-mineral preparation. Data generated from pharmaceutical, analytical studies can be used to develop a preliminary standard profile for the formulation of Trinetra Ras.

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