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Research Article

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

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ABSTRACT

Trinetraras is a *Kharliya Rasayan* mentioned in *Yogratnakar* for the treatment of *Hridroga* (*Yogratnakar Uttarardha Hridroga Chikitsa* 1,2). It consists of *Shuddha Parad*, *Shuddhagandhak* and *Abhrakbhasma* where Bhavanadravya is *Arjuna* bark decoction. **Aim:** Pharmaceutical and analytical study of *Trinetraras*. **Materials and methods:** *Paradshodhan, Gandhakshodhan, Vajrabhrakshodhan, Dhanyabhraknirmana,* preparation of *Abhrakbhasma*, preparation of *Kajjali* are the procedures required to be performed before the preparation of *Trinetraras*. Total 40 times *Abhrak* was subjected to *Maransanskar* in *Gajaputa* (40 puti *Abhrakbhasma*). *Trinetraras* can be prepared from *Shuddha Parad, Shuddhagandhak* 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 *Trinetraras* 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 *Trinetraras*. **Conclusion:** 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 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 *Trinetraras*.

Trinetraras 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*. *Trinetraras* is one of them. *Trinetraras* 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*).

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 *Ras-shastra*.
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 *Trinetra 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

Ashuddha Parad	-	500 gm
Kumariswaras	-	100 mL
Chitrakamoola	-	100 gm
Raktasarshapa	-	100 gm
Brihati	-	100 gm
Triphala	-	100 gm

Water for *Kwath* preparation - 3.2 lit

Total *Kwath* prepared - 0.8 lit

Procedure

Chitrakamoola, 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 *Khalvayantra* 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

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

Ingredients:

1) *Ashuddha Gandhaka*	-	500 gm
2) *Goghrita*	-	500 gm
3) *Godugdha*	-	2 lit
4) Luke warm water		-q.s.

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

Ashuddha Abhraka	- 500 gm
Triphala Kvatha	-1000 mL.

Procedure

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

few minutes the *Triphala Kvatha* 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*[4]**

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*[5]

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

Ingredients

*Dhany*abhrak-200 gm

Jaggary -200 gm

Erandapatraswaras- q.s.

Procedure

Dhanyabhrak 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 *Sharavasamputa* were shed dried and subjected for *Maranasanskara*. In cuboidal pit cow dung cakes were filled 2/3 then *Sharavasamputa* 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*[6]

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

Ingredients:

Shuddha Parada	- 200gm
Shuddha Gandhaka	- 200gm.

Procedure

Shuddha Parada was taken in a Khalvayantra; equal amount of *Shuddha Gandhak*a 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*[7]

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

Name of the procedure: *Bhavanasanskar* (Trituration)

Equipments: weighing machine, measuring jars, *Khalvayantra*

Ingredients

Shuddha Parad-50 gm

Shuddha Gandhak-50 gm

Abhrakabhasma-50 gm

Arjuna bark decoction- 200 mL

Procedure

Kajjali was prepared by taking equal amount of *Shuddha Parad* and *Gandhak*. *Abhrakabhasma* 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.

***Sookshma (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 *Shlakshnatva

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 CS_2, H_2O, Dilute HCL, Moderate concentrated HNO_3 and Aquaregia.

Successive solubility of *Abhrakabhasma* was carried out in H_2O, Dilute HCL, Moderate concentrated HNO_3 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-Ciocalteau reagent.

Thin Layer Chromatography

It was run on silica gel G-254 F (Merck)in solution Tolune: 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 morter. 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 bhraka* was brittle in nature with decreased lustre.

Results

Samples	A-1	A-2	A-3
Initial weight	500 gm	500 gm	500 gm
Final Weight	465 gm	460 gm	456 gm
Weight loss	35 gm	40 gm	44 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*.

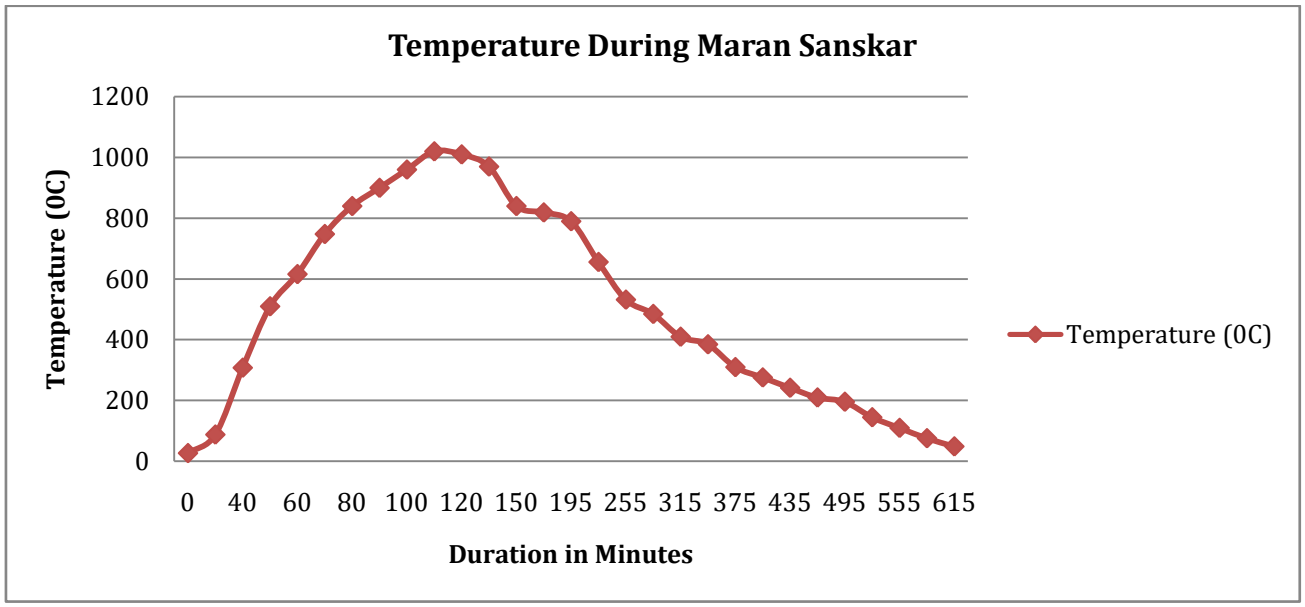
Results

Samples	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 *Sindhurabha* 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*. After 30 hours of *Mardana*, *Kajjali* became *Nischandra, Sookshma, Shlakshna, Rekhapurna* and *Varitar*.

Results

Samples	K-1	K-2	K-3
Initial weight	400 gm	400 gm	400 gm
Final Weight	380 gm	374 gm	370 gm
Weight loss	20 gm	26 gm	30m

Preparation of *Trinetraras*

Showing observations of preparation of *Trinetraras*

Samples	T.R.-1	T.R.-2	T.R.-3
Initial weight	150gm	150gm	150gm
Final weight	164gm	162gm	161gm
Weight gain	14gm	12gm	11gm

Analytical Study

Ayurvedic Parameters

Ayurvedic parameters for *Bhasma pariksha* revealed the results as follows

Prepared samples of *Abhrakabhasma* (AB-1, AB-2, AB-3) were *Nishchandra, 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	o.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 H_2O (%)	In Dil.HCl (%)	In Mod. Conc.HNO_3 (%)	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 CS_2 (%)	In H_2O (%)	In Dil.HCl (%)	In Mod. Conc.HNO_3 (%)	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)	-		

7	0.3357	+ (Brown)	+ (Very faint)		
8	0.4142	+ (Brown)			
9	0.07142			+ (Blue)	+ (Blue)
10	0.1142			+ (Blue)	+ (Blue)
11	0.2857			+ (Blue)	+ (Blue)
12	0.6571			+ (Blue)	+ (Blue)

DISCUSSION

Paradshodhan 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 *Ghruta* was floated on the surface of milk which clarifies the significance of *Gandhan shodhan*.

Abhrakashodhan was done as per the reference of R.R.S.2/16-17 by quenching the *Abhraka* pieces into *Triphalakvath*. The procedure was repeated for 7 times and observations were noted. Large sized Vajr*abhraka* get converted into smaller sized pieces of *Vajrabhraka*. Shodhit *Abhraka* was brittle in nature with decreased lustre. *Dhanyabhraka* was prepared according to the reference of R.S.S. 1/154. The prepared *Dhanyabhraka* was uniformly fine powdered form with decreased lustre. The procedure typically resembled as stated in the literature.

Preparation of *Abhrakabhasma* was carried out as per the reference of R.R.S.2/26-27. Jagary was added in equal proportion to that of *Dhanyabhraka*. This Jagary act as an oxidizing agent in the incineration. Total 40 times *Abhraka* was subjected to *Maransanskar* in *Gajaputa*. At the end *Sindurabhabhasma* 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.Hridrog 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; *Nishchandratv, Sindurabhavarn, Rekhapurnatva, Sookshma, Sparshakomal (Mrudutva/Shlakshnatva), 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 *Abhrakbhasma* 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. *Abhrakbhasma* 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 *Abhrakbhasma* were maximum soluble in dil.HCl and insoluble in mod.conc. HNO3 and aquaregia 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 *Abhrakbhasma* 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 Abhrakbhasma* 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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