Dasari Srilakshmi and Prasanna Lakshmi. / *International Journal of Biological & Pharmaceutical Research.* 2016; 7(2): 83-93.

e- ISSN 0976 - 3651
Print ISSN 2229 - 7480

International Journal of Biological & Pharmaceutical Research
Journal homepage: www.ijbpr.com

IJBPR

AN ANALOGOUS STUDY OF PHARMACEUTICO-ANALYTICAL ASPECTS OF TAL SINDOOR AND SHILA SINDUR

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ABSTRACT

Parada (mercury) and Gandhaka (sulphur) are the chief materials used in Rasa Shastra. Kupipakwa yogas (sublime product formed from mercury and sulphur) are one of four Murchita parada yogas. Tal Sindoor (TS) and Shila Sindur (SS) are prepared with Suddha parada (purified mercury), Suddha gandhaka (purified sulphur), Suddha haratala (purified orpiment)/Suddha manahshila (purified realgar) respectively. TS and SS are prepared with $Hg+S+As_2S_3$ and $Hg+S+As_2S_2$ respectively as ingredients. Though these compounds contain same ingredients, mode of preparation and similar indications, but they vary with a difference of one sulphur molecule in chemical form for which they greatly vary in their clinical applicability, efficacy and dosage. The present study was conducted to evaluate the comparative pharmaceutical and analytical aspects between TS and SS. TS and SS were prepared by adopting similar temperature pattern. Four samples each of TS and SS were prepared. The comparative observations, precautions and physical properties of the prepared samples were recorded. Evaluation of observations of pharmaceutical procedure revealed that during preparation of SS excessive accumulation of sulphur near the neck of Kachakupi (glass bottle) was observed, prolonged stage of fumes and strong typical odor of arsenic, fuel consumption, manual labor and total duration of all samples of SS were relatively more than respective samples of TS. Intermediate product Kajjali (collyrium like product resultant of triturating Hg and S) and final products TS and SS were subjected to organoleptic test as per Ayurvedic science, qualitative and qualitative analysis with modern parameters. Both products were prepared according to classics, the hygienic conditions maintained during preparation was monitored by evaluating microbial contamination. Qualitative and quantitative analysis of TS and SS were similar. Microbial load of infectious species were absent. Hence both TS and SS were prepared under hygienic conditions, and preparation of TS was easy and safe compared to SS.

Key Words: Kupipakwa rasayana, Pharmaceutico-analytical study, Shila Sindur, Tal Sindoor.

INTRODUCTION

Ayurveda is science of life implicates laws of nature to maintain and regain health. Centuries ago our planet was rich with flora, in due course of time, desired plants became scarce. Hence an alternative was searched and medicines were prepared from metals and minerals for therapeutic benefit and metallic pharmaceutical preparations which are comparatively stable, quick in action, rich in potency, lesser dosage and longer shelf life. Kupipakwa rasayana (Jha CB, 2007) is a unique pharmaceutical preparation where in the drug is prepared in a glass bottle called Kachakupi and the processing is done in a traditional furnace with a pattern of gradual rise in temperature. Kupipakwa yogas have mineral and metallic formulations including both Sagandha (presence of sulphur) and Nirgandha (absence of sulphur) prepared with mercury as ingredients. TS and SS are Sagandha, Sagni (with heating process) mercurial preparation

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processed by Kupipakwa method. Both are Kantastha kupipakwa rasayana (final product collected near neck of the bottle) which are best and fast acting arsenic medicines, having mercury, sulphur, and arsenic tri suphide/arsenic di sulphide respectively as ingredients mainly used in aliments like Kustha (a group of skin disorders), Shwasa (respiratory problems including bronchial asthma), Sannipataja roga (disease caused due to involvement of 2 of three bodily humors) and helps in rejuvenation. Physical and chemical properties help develop quality in the final product. This is one of the essential aspects to evaluate the fixed standards of physicochemical features to ensure the desired action, due to wide diversity of identification and manufacturing procedures. It was felt necessary to evaluate the induction of new physicochemical properties of these drugs by their unique method of preparation and can be investigated scientifically through chemical analysis.

Antiquity

The preparation of Sindura kalpana can be traced to 12th century A.D, but TS and SS had been introduced in the early years of 20th century as indicated in the classical texts 'Rasendra sambhava', 'Basavaraajeeyam', 'Rasayana Sara', 'Siddha Bheshaja Manimala' and 'Rasamruta'.

Etymology

Etymologically, the word Sindhura (Monier MW, 2002) is derived from the root 'Syanda prasravane' means 'releasing properties'. It was also explained by different authors pharmaceutically as a process which converts various metals like mercury etc ingredients to red coloured substances. Pharmacologically it may be explained as the substance which acts quickly on deep seated vitiated Doshas (three energies believed to circulate in the body and govern physiological activity, their differing proportions determining individual health or aliments) and eliminates Doshas from the body. Due to ingredients Haratala/Tala and Manahshila/Shila and the final product being vermillion coloured i.e. Aruna (Sindura) varna, hence they are known as Tal Sindoor and Shila Sindur respectively.

MATERIALS AND METHODS

Major materials

Parada (Vagbhatta A, 2003a), Gandhak (Vagbhatta A, 2003b), Haratala (Vagbhatta A, 2003c), and Manashila (Vagbhatta A, 2003d), were the raw materials used in the study. They were procured according to the acceptable characters mentioned in the classics and authenticated by experts.

Associated materials

Kumari (Aloe vera Tourn. Ex Linn.) (Sharma PV, 1999a), Arka (Calotropis procera Aiton.) (Sharma PV, 1999b), Haridra/turmeric (Curcuma longa Linn) (Sharma PV, 1999c), cow's milk, Ardraka (Zinzeber officinale Roscoe) (Sharma PV, 1999d), Kushmanda (Benincasa hispida Thunb.) (Sharma PV, 1999e), are procured for purification process of raw drugs.

Equipments required

Khalva yantra (mortar and pestle) was used for purification of mercury and arsenic di sulphide. Pathana yantra was used for purification of sulphur; Dola yantra was used for purification of arsenic tri sulphide. Valuka yantra was used for preparation of TS and SS.

Pharmaceutical processing

All the pharmaceutical processes were carried out in Department of Rasashastra, JSS Ayurveda Medical College, Mysuru, India. They include following steps – sodhana of raw drugs, preparation of kajjali, kajjali with haratal and kajjali with manahshila, and preparation of TS and SS.

Sodhana of raw drugs

Purification of mercury (Sri Bhatta Gopala Krishna, 2003) was done by triturating with turmeric powder and Kumari swarasa (Aloe pulp). This paste was made into round, small flat pieces (Chakrikas) dried in shade and subjected to Urdhwapatana (sublimation). On self-cooling, purified Parada was collected from inner surface of upper pot. Gandhaka shodhana (sulphur purification) was done with cow's milk by Bhudhara puta method (Vagbhatta Acharya, 2003e) Later purified Gandhaka was washed with warm water and collected. Haratal purification was done by Swedana in Dola yantra (type of instrument) with Kushmanda Swarasa (Benincasa hispida's fruit pulp juice) (Vagbhatta Acharya, 2003f) Purification of Manashila (Vagbhatta Acharya, 2003g) was done by triturating with ginger juice for seven times. [Plate:1]

Preparation of kajjali

Equal quantity by weight of purified mercury and purified sulphur were taken in mortar and pestle (Khalva yantra) to prepare Samaguna kajjali (Hg+S). They were triturated for about 38 hours till Kajjali siddha lakshanas (Vagbhatta Acharya, 2003h) (complete formation of Kajjali) were attained. Samaguna kajjali was triturated along with Haratala ($Hg+S+As_2S_3$) and further one third quantity was triturated with Arka ksheera (Pandit Dwivedi Vishwanath, 1997) to prepare sample KH1 and remaining two thirds part of Kajjali was triturated with Kumari Swarasa (Vaidya Pandit Sharma Hariprapanna, 1983). to prepare sample KH2. Purified Manahshila was triturated with Samaguna kajjali to form Kajjali ($Hg+S+As_2S_2$). Kumari swarasa (Vaidya Yadavji Trikamji, 1998; Vaidya Pandit Hariprapanna Sharma, 1983) was added and triturated to prepare sample KS.

Preparation of TS and SS

After drying above prepared samples Kajjali was filled in Kachakupi (glass bottle) covered with seven consecutive layers of cloth smeared with Multani mitti (fuller's earth) up to mouth of Kachakupi and placed in Valukayantra (apparatus designed with iron vessel filled with sand). After the entire apparatus was ready, wood was set to fire, following gradual heating pattern. Hourly temperatures near the base of glass bottle were recorded with pyrometer. The temperature was maintained between $150^{o}C - 250^{o}C$ for mild heat, raised to $350^{o}C - 600^{o}C$ for moderate heat and intense heat up to $750^{o}C$. After the stage of fumes and flames, the bottom of the bottle appears like rising sun that is red in colour (Udayabhaskara varna). After confirming with copper coin test, bottle was corked and intense heat was continued for 2 hours. Later Kachakupi was left for self-cooling, bottle was removed from Valukayantra. The cloth with Multani was scrapped off and bottle was broken 2 inches below the collection of the TS. Later the drug was collected by tapping over the outer surface of glass bottle (Shri Harisharanananda Ji, 2000; Vasudev MD, 1997; Joshi Damodhar, 2000). Same procedure was repeated to prepare three other samples of TS and four samples of SS.

Collection of final product

Four samples each of TS and SS were prepared, first trail of Tal Sindoor (TS1) and Shila Sindur (SS1) were done in glass bottle of 650ml capacity. Second trail TS2 and SS2, were conducted in 350ml capacity bottle. Sample 3 and 4 of each i.e. TS3, TS4 and SS3, SS4 were prepared in two bottles of 350ml capacity, in a single trail to check efficacy of fuel, time and output of both TS and SS. [Plate:2]

Analytical study

Analytical study was conducted with a view to know particular chemical configuration of intermediate and final products. It includes qualitative and quantitative analysis. Qualitative data included organoleptic characters and qualitative tests for identification of heavy metals and minerals. Organoleptic characters of final products are as follows TS1 was dark brownish red in colour where as the remaining three samples of TS are brownish red in colour. All four samples of SS are brick red in colour. Four samples each of TS and SS are smooth on outer surface and after powdering the final product, TS and SS are soft smooth powder, tasteless and odourless.

To check solubility 10 ml of each solvent is taken separately in a test-tube. Then 100 mg of each sample of drug is added in taken solvent. Then it is shaken for every 15 minutes up to 12 hours. After 12 hours, solubility was observed. Estimation of microbial contamination was done by the ‘plate count method (Pharmacometric, 1964). Assay of mercury, sulphur and arsenic were done to estimate the quantity of respective material in intermediate and final products [Plate:3].

OBSERVATIONS AND RESULTS

Pharmaceutical study

After 17 hours of triturating mercury with turmeric, the resultant colour was dark greenish yellow, mercury disintegrated into tiny globules. Later Aloe pulp was added to attain a homogeneous mixture of mercury, turmeric and Aloe pulp that was subjected to sublimation. Thin layer of shiny grey coating was observed on inner surface of the upper pot. After purification of sulphur, bright yellow crystal was changed to pleasant yellow small beads, with reduced pungent odour. No physical changes were observed before and after purification of Haratal. After purification of Manashil coarse powder was changed to fine dull orange colour powder, with strong pungent ginger odour. Shiny liquid mercury was triturated with purified beads of sulphur that changed to grey coloured powder and after triturating for 36 hours, uniform collerium like Kajjali was prepared that attained all Samyak siddha kajjali lakshanas [Table 1].

Common observations of each trail

Different phases of the desired characteristics during the process were observed namely sulphur fumes, melting and boiling of Kajjali, stage of flames and confirmative test like disappearance of flame, Sheeta shalaka (cold thin iron rod) test, red hot appearance of bottom of the bottle and copper coin test were observed and recorded in all four trails each of TS and SS [Chart 1]. Final product was collected from the neck of Kachakupi from all the batches of TS and SS. They were weighed and calculated for the percentage of absolute and relative yield [Table 2]. Final product SS1 had yellow crystals over the product. Hence following three trails of SS and four trails of TS were heated for an extended period of 10-15 minutes after copper coin test and before cooking. Yellow crystal collection was absent in remaining seven samples. Output of final product in all the 8 samples was around 50-60%. They were subjected to various organoleptic [Table 3] and physiochemical parameters [Table 4-Table 8].

Comparative observations of each trial

Lumps of Kajjali were formed due to gummy latex of Arka ksheera. Giving Bhavana was very difficult; hence Kajjali was left for drying in mortar. KH1 took longer time for drying compared to KH2. KH1was in granule form and that of KH2 and KS was in fine powder form. The initiation of sulphur fumes and all other phases were observed earlier in TS compared to SS with their respective trails. Strong arsenic and sulphur odour was observed in all trails of SS compared to respective trails of TS during the stage of fumes. Blockage at the neck portion of the bottle was more in case of SS when compared to TS. Stage of flames was comparatively more in SS with respective TS, except TS1. The duration of stage of flames was 1 hour 35 minutes in TS1 and all other samples had only for duration of 45 minutes to 1 hour. After stage of

flames, it took longer time for SS trails to clear the residual sulphur fumes compared to respective trails of TS.

Precautions

- Bhavana (lavigation) was done with sufficient quantity of Drava dravya (liquid media) and consistent pressure.
- The base of the bottle was pasted with Multani mitti to avoid air gap. Compact Multani mitti application was done and allowed for drying after each covering.
- Temperature was maintained at different phases as described in classical texts to obtain optimum quantity of final product (50-60%).

Table 1. Results of the purification of raw drugs

Raw drug-trail	Duration of purification process	Quantity of raw drug before purification	Quantity of raw drug after purification	Loss (gm)	% of out put
Parada-trail I	6 hrs	250 gm	238 gm	12	95.2
Parada-trail II	6 hrs	250 gm	240 gm	10	96
Gandhaka-I	30 mins	250 gm	246 gm	4	98.4
Gandhaka-II	30 mins	250 gm	245 gm	5	98
Haratal	3 hrs	150 gm	147	3	98
Manashila		250 gm	273 gm	23(gain)	109.2
Kajjali-I (Hg+S)	37 hrs	130(Hg)+130(S)	257 gm	3	98.8
Kajjali-II (Hg+S)	36 hrs	130(Hg)+130(S)	255 gm	5	98.1
Kajjali-III (Hg+S)	10 hrs	Above kajjali-520 gm	512 gm	5	98.46
KH1	30 mins Later left for drying	150 gm	160 gm	10(gain)	106.6
KH2	6 hrs Later left for drying	225 gm	233 gm	8 (gain)	103.6
KS	6 hrs Later left for drying	375	382	7 (gain)	101.9

Table 2. Comparative observation and results of 4 trails of TS and SS

Observations (duration in hours)	TS1	TS2	TS3	TS4	SS1	SS2	SS3	SS4
Stage of mild heat	8:25	6:45	5:50	05:50	10:00	07:05	6:10	6:10
Stage of moderate heat	06:40	04:15	03:50	03:50	07:35	05:20	04:45	04:45
Stage of intense heat	04:40	04:25	04:00	04:00	05:10	04:45	04:00	04:00
Total duration	19:45	15:25	13:40	13:40	22:45	17:10	14:55	14:55
Time for selfcooling	20	17	16	16	24	18:30	16	16
Time taken from commencement of process for stage of flames	14:00	10:30	08:40	08:40	17:50	11:45	10:10	10:00
Time taken from commencement of process for copper coin test	18:00	13:30	12:30	12:30	20:45	15:15	13:00	13:00
Quantity taken for final product in grams	135	90	60	60	135	90	60	60
Bottle capacity in ml	650	350	350	350	650	350	350	350
Quantity of sample obtained	76 gm 56.29%	53gm 58.89%	34 gm 56.67%	35gm 58.33%	83gm 61.48%	51gm 56.67%	30gm 50.0%	32 gm 53.33%

Analytical study**:**

Table 3. Comparative Ayurvedic tests

Tests	Kajjali	TS1	TS2	TS3&TS4	SS1	SS2	SS3&SS4
Rekhapurnatva	+	+	+	+	+	+	+
Nischandrikatva	+	NA	NA	NA	NA	NA	NA
Varitaratva	+	NA	NA	NA	NA	NA	NA
Tamra pareeksha	+	NA	NA	NA	NA	NA	NA
Kathorata	NA	+	+	+	+	+	+

Table 4. Comparative physicochemical analysis of Kajjali and final products

S.No	Tests	KH	KS	TS 1	TS2	SS1
1.	P^{H}	6.85	7.39	7.34	7.53	7.41
2.	Moisture content	0.52	0.42	0.43	0.49	0.48
3.	Acid insoluble ash	74.66	75.36	60.73	50.48	68.11
4.	Total ash	1.11	1.64	1.09	1.64	1.64
5.	Alcohol soluble extract	29.76	57.50	29.77	48.85	19.94
6.	Water soluble extract	9.95	49.67	4.98	19.44	9.92
7.	L.O.D at 105^0C	0.52	0.42	0.43	0.49	0.48

Table 5. Comparative chemical analysis of processed, intermediary and final products

S.No	Sample Name	Moisture content & Loss on drying	Solubility – Con.HCl	Solubility – Glacial Acetic acid	Solubility – Honey
1.	Su.Gan	0.8% w/w	Insoluble	Insoluble	Partially soluble
2.	Su.Ha	0.4% w/w	Insoluble	Insoluble	Partially soluble
3.	Su.Ma	0.9% w/w	Insoluble	Insoluble	Partially soluble
4.	Kajjali	0.3% w/w	Sparingly soluble	Sparingly soluble	Partially soluble
5.	KH1	0.6% w/w	Sparingly soluble	Sparingly soluble	Partially soluble
6.	KH2	0.4% w/w	Sparingly soluble	Sparingly soluble	Soluble
7.	KS	0.4% w/w	Soluble	Sparingly soluble	Soluble
8.	TS1	0.5% w/w	Partially soluble	Sparingly soluble	Soluble
9.	TS2	0.4% w/w	Partially soluble	Sparingly soluble	Soluble
10.	SS	0.5% w/w	Partially soluble	Sparingly soluble	Soluble

Table 6. Solubility test of Kajjali and final products

S.No	Media	KH	KS	TS1	TS2	SS1
1.	Distilled water	Insoluble	Soluble	Partially soluble	Partially soluble	Partially soluble
2.	HCl	Insoluble	Soluble	Partially soluble	Partially soluble	Partially soluble
3.	Chloroform	Soluble	Soluble	Soluble	Soluble	Soluble
4.	CCL_4	Soluble	Soluble	Soluble	Soluble	Soluble
5.	Isopropyal alcohol	Partially soluble	Soluble	Soluble	Soluble	Soluble
6.	Petroleum ether	Soluble	Soluble	Partially soluble	Partially soluble	Partially soluble
7.	Glycerin	Insoluble	Insoluble	Insoluble	Insoluble	Partially soluble
8.	Benzene	Soluble	Soluble	Soluble	Soluble	Soluble
9.	Acetone	Partially soluble	Soluble	Soluble	Soluble	Soluble
10	CH_3OH	Partially soluble	Soluble	Soluble	Soluble	Soluble
11	Methylenedichloride	Soluble	Soluble	Soluble	Soluble	Soluble
12	Ether	Soluble	Soluble	Partially soluble	Partially soluble	Partially soluble

Table 7. Estimation of microbial contamination of Kajjali and final products

S.N	Variety of Microbes	KH2	KS	TS1	TS2	SS1
1.	Total Bacterial Count (CFU/ gm)	3300	1400	2100	3100	3900
2.	Total Fungal Count (CFU/ gm)	40	30	40	40	40
3.	E.Coli	Absent	Absent	Absent	Absent	Absent
4.	Salmonella	Absent	Absent	Absent	Absent	Absent
5.	P.aeruginosa	Absent	Absent	Absent	Absent	Absent
6.	S.aureus	Absent	Absent	Absent	Absent	Absent

Table 8. Assay of mercury, sulphur & arsenic of Kajjali and final products

S.No	Ingredients	KH1	KS	TS1	TS2	SS1
1.	Mercury	30.65%	31.08%	61.02%	58.80%	59.11%
2.	Arsenic	19.12%	20.75%	14.75%	16.02%	13.86%
3.	Combined sulphur	21.73%	22.69%	18.98%	19.13%	19.75%
4.	Free sulphur	23.09%	21.34%	3.24%	4.25%	4.70%

Fig 1. Temperature Graph Of All Trails Of SS And TS

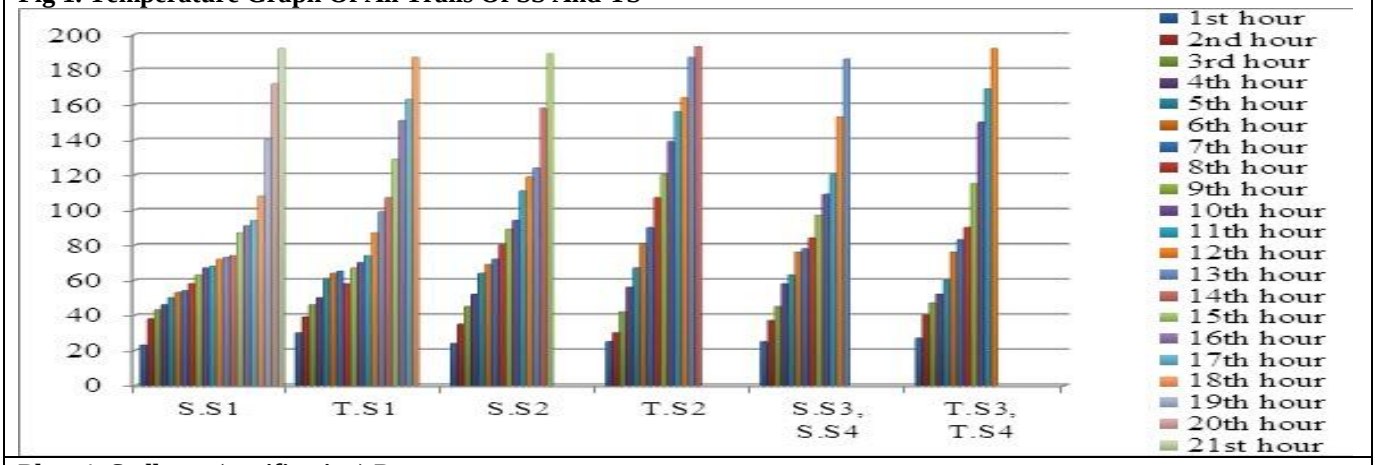

Plate 1. Sodhana (purification) Process

Plate 2. Preparation of TS

Plate 3. Preparation of SS

Plate 4. Chemical tests for intermediate and final products

DISCUSSION

Purification of mercury was done by triturating with turmeric powder that may decrease the surface tension leading to disintegration of mercury molecules. While triturating with Aloe pulp, the small globules of Parada disintegrated completely to form a uniform batter, this may be due to binding of mercury, turmeric and Aloe. This avoids spillage of Parada when Chakrikas were prepared or transferred to the pot for sublimation. Remaining impurities of Parada may be separated during sublimation, since Hg will evaporate at the temperature of 356°C, turmeric and Aloe pulp along with other organic and inorganic impurities like Naga (lead) and Vanga (tin), have high boiling point and cannot sublimate with Hg. The active principle, cucumin might have reacted with the impurities, especially with mercury salts present along with mercury and help in removal of mercury salts, thus purifying Parada chemically. Parada thus obtained was brighter compared to the raw drug, which indicates the absence of physical impurities it matches with tests described in the classics, which may indicate the absence of physical impurities like soot, etc. The loss of amount of purified mercury can be during the process of triturating, sublimation and collection of purified mercury.

Gandhaka purification mainly serves 3 purposes, purification, detoxification and potentiating therapeutically. During the process, when temperature reaches 115^{o}C, sulphur melts and dribbles down into the milk in the form of granules leaving behind the physical impurities like mud, stones etc., on the cloth. Since these impurities don't change at this temperature. This can be considered as purification. Sulphur is available in combined state along with copper, lead, zinc, iron, lime stone etc., which are unwanted that retain on the cloth at 115^{o}C. Even if these toxins pass through the cloth and fall into milk, the fat content of milk which in the form of micro globules will remove the fat soluble impurities. Melted sulphur comes in contact with milk and solidifies preventing chances of remixing of impurities settled at the bottom. Purification of sulphur is similar to sublimation followed by granulation. When sulphur was heated, molten sulphur passes through the cloth and gets condensed in the milk. Since milk has fatty acids and lipids, this helps condensation of sulphur. Through sublimation, granulation can be achieved by melting sulphur and passing through a

small hole and cooling. The vapors of sulphur reach the bottom of the collecting flask and solidify. Sublimation always purifies the crude products. Hence above purification process, purifies sulphur chemically and enhances the properties of milk.

Purification of Haratala facilitates continuous contact and uniform indirect heating pattern. Pumpkin juice has (-)-curcurbetine which might be removing some oxide and other arsenic compound impurities associated with As_2S_3 and it may not be reacting with pumpkin juice. Thus after purification process there was no appreciable external change in Haratala. During purification of Manashila, the quantity of Ardraka swarasa for Bhavana reduced after 3rd Bhavana and remained constant till 7th Bhavana. This might indicate absorption of moisture content initially and hence the quantity of ginger juice might have reduced in the consecutive Bhavana. There was colour change in Manashila from bright reddish orange to orange colour, which might be due to chemical reaction. Active principle in Ardraka swarasa are 'gingerol' {(S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-3-decanone} and shogaol {(E-1-(4-Hydroxy-3-methoxyphenyl) dec-4-en-3-one)}. Shogal is the reason for pungent odor in ginger. There could be weak interaction between As_2S_2 and gingerol and shogaol. The weight increase is due to the absorption of organic molecules Ardraka sattva/starchy portion into powder of As_2S_2.

Mercury belongs to IIB group, it can give away 1 or 2 electrons to form a bond, arsenic belongs to VA group and can share by giving away 3 electrons forming As+3. Sulphur belongs to VIA group and can share by taking 2 to 6 electrons forming S-2 to -6 valences. Both arsenic and mercury have more affinity towards sulphur to form sulphide compound. Sulphur belongs to thiolic group which has more affinity to mercury to form its sulphide, these two groups counter act each other's action. Sulphur is considered as an antidote for mercury poisoning and thus this combination may result in making an inert substance called Kajjali. According to Goodman-Gillman pharmacology mercury readily forms covalent linkages with sulphur. When sulphur is in the form of sulphydryl group, divalent mercury replaces the hydrogen atom to form merceptides, X-Hg-SR and Hg(SR)2 where 'X' is an electronegative radical and 'R' is protein. Sulphydryl compounds have long been called 'merceptans' because of their ability to capture mercury. Mercaptan=A Sulphur-containing organic compound with the general formula RSH where R is any radical, especially ethyl mercaptan C2H5SH also called thiols (mercurium captans=a substance seizing mercury.) The affinity of mercury for thiols provides the basis for treatment of mercury poisoning by such agents as dimercaprol and penicillamine. The minerals and metals when processed with addition of sulphur or sulphur containing minerals immediately get converted into sulphides and most of these are non-toxic. Cu, Pb, Sn, Zn, As etc; are highly toxic if they are in oxide form, but their sulphate compounds are nontoxic or least toxic. Arsenic always has affinity towards oxygen and sulphur, so may form weak bond with excess sulphur in Samaguna Kajjali (Hg+S). On long exposure to light, realgar disintegrates into a reddish yellow powder (As_2S_3). Arsenic also has a formal oxidation state of +2 in As_4S_4, realgar. This is achieved by pairing 'As' atoms to produce dimeric cations $[As\text{-}As]^{2+}$, so the total covalence of 'As' is still three.

The gradual colour change of Kajjali from ash colour to complete black suggests the gradual process of amalgamation. The absence of luster may indicate the physical absence of free mercury. When Hg treated with S, the resultant product is HgS. This can be in two forms (alpha-red, beta-black). The black colour of Kajjali might be because of formation of black sulphide of mercury. Fine powder of purified Haratala was triturated with Samaguna Kajjali (Hg+S) to facilitate even mixing of Kajjali ($Hg+S+As_2S_3$) and reacting with free sulphur. There might be weak bonding in this process. Movement of pestle was very difficult may be due to gummy resins in Arka ksheera. Wet trituration helps in attaining homogeneous mixture than dry trituration. It acts as a binding agent that may help in further complex bonding of Kajjali. The alkaloids present in Kumari get bonded with Samaguna kajjali with Haratala and Manashila that might help to increase the efficacy of the TS. Kajjali gained weight of 8, 10 gm after the Bhavana with Arka ksheera and Kumari swarasa in KH1, KH2 and KS respectively may be due to addition of organic matter.

The objective of seven layers of Mrith lepana for Kachakupi is to strengthen it for sustained heating by preventing breaking of Kachakupi in between the process. A lump of Mrith lepana was applied at the base of the bottle before wrapping, so as to avoid the air gap. Only lower 1/3rd of the Kachakupi was filled with the drug, to provide space inside the bottle for melting, boiling and sublimation of Kajjali inside the bottle, else large quantity may hinder the sublimation and may lead to over flow of boiling Kajjali from the mouth of Kachakupi during the process. Valuka yantra is specially designed for distribution of uniform indirect heat, which may prevent temperature fluctuation of the Kachakupi. Valuka (sand) is inert and it may render resistance to the apparatus from atmospheric temperature variations.

Detail description of TS and SS were not enumerated in classical texts, hence normal Kupipakwa procedure was followed. There are also references of TS and SS named as 'Chandrodayas' by the text Rasayana sara, but ingredients and mode of preparation are similar. Availability of references of TS is more compared to SS. TS and SS were prepared by heating Parada with Gandhaka and Hartala/Manashila which may help to exhibit superior qualities compared to other formulations. All trials of SS were prepared with Kajjali KS and that of TS1 with KH1 and for TS2, TS3 and TS4 with KH2. But

there was a variation in the quantity taken and hence different capacity bottles were used. The most common problem while preparing TS and SS was blocking of the Kupi due to sulphur fumes this was more in case of all trails of SS than respective TS. This might be due to dissociation of Manashila and releasing free sulphur as this was natural course. This problem was overcome by filling the sand up to the brim of Kachakupi, by which the adherence of fumes was reduced to maximum extent.

The temperatures recorded at the base level of the Kachakupi indicate the temperature at which the drug is being processed. Though there was association of arsenic with Gandhaka during the stage of fumes in all samples of TS and SS, there was prolonged stage of fumes with strong odour of arsenic during the process of SS with more orange-yellow fumes deposited on Shalaka. Appearance of sulphur fumes after 2 hours of heating indicates the melting of Kajjali which was confirmed by Sheeta shalaka test. Appearance of dense and profuse fumes is suggestive of melting of Kajjali and processing of Parada with Gandhaka. Appearance of flames may indicate the burning of extra sulphur and organic matter. The haziness was observed after stage of flames, the bottom of Kachakupi couldn't be seen clearly when viewed with torchlight. This may be due to vapours of sulphur as the high temperature might inevitably cause the evaporation of sulphur before coming in contact with oxygen. Copper coin test may help to confirm absence of free sulphur and escaping of white mercury fumes. Loss in final product compared to that of Kajjali taken may be due to the loss of major proportions of sulphur, minimum quantity of arsenic and traces of mercury. Before corking, yellowish orange and red particles were observed in the middle portion of the bottle. This might be due to presence of arsenic along with mercury and sulphur.

Completeness of preparation was confirmed by following observations like absence of flame may indicate completeness of burning organic and excess sulphur. Cessation of fumes might indicate complete escape of excess free sulphur. Copper coin turned greyish white which was placed over the mouth of the Kachakupi, this may be due to reaction of mercury with copper. When cold Shalaka was sent in Kachakupi without touching the sides of bottle, yellowish orange layer was formed over the Shalaka indicative of deposition of fumes of TS/SS which is due to presence of arsenic and no remnant material was adhered to the tip of Shalaka. These tests confirms the completion of Sindura preparation and suitable for corking of the bottle. Appearance of redness in the bottom of Kachakupi "Arunachandrodaya varna", might suggest complete sublimation of Kajjali and the visibility of red hot bottle bottom. Before corking, reddish yellow shining particles were observed below the neck of bottle, which might be the initial phase of condensation of the fumes of the TS/SS.

For collection and storage of final products, Kachakupi was removed from sand and then outer multani layer was removed after self cooling to avoid mixing of unwanted sand material in medicine. Kachakupi was broken by burning thick thread dipped in kerosene which causes sudden increase in temperature around glass bottle. After thread had burned totally, bottle was covered with wet cloth. Due to sudden fluctuation in temperature, glass bottle breaks as it cannot accommodate sudden variation in temperature. This method of breaking helps heating glass bottle and avoids small pieces while breaking. SS was more reddish where as TS was reddish brown colour, may be due to presence of realgar and orpiment respectively. The change in black colour of Kajjali to red coloured Sindhura after the heat contact may be due to change in their allotropic form and re-arrangement of crystalline structure. Orpiment and realgar vary with one sulphur atom vary in colour from yellow to red. Similarly the slight change of colour of TS and SS might be due to the amount of arsenic and sulphur exhibiting variation in colour and crystal lattice structure of final products.

Ayurveda have described subjective organoleptic parameters. The ancient parameters to evaluate Kajjali, TS and SS were positive, suggestive of following appropriate methods of preparation. Kajjali obtained after trituration was black fine powder that was Slakshna (smooth), Sukshma (subtle) that could pass Rekhapurna (filled in furrows of fingers) that may denote the fineness and reduced particle size to enhance bioavailability. Nishchandratva (absence of luster) and Tamra pareeksha (rubbing Kajjali over Copper foil) may denote the absence of free mercury in Kajjali. Varitara Kajjali (floating on water) indicates the lightness, though both mercury and sulphur are heavy to sink in water, but after processing it could float on water. The final products TS and SS were Katora (hard) and solid mass (Sindura). When rubbed against paper red colour streaks were observed indicative of presence of mercuric sulphide in it.

Modern parameters like the P^H values of KH1, KS when compared with TS1, TS2 and that of SS1 respectively, results suggested gradual increase towards alkalinity. All the values indicate the partial alkalinity of the drugs which might be due their Panchabhauthic (five elements) composition of ingredients of the drugs. As the drug is alkaline in nature, the inference can be made that they may not precipitate gastric irritability. Loss on drying and moisture content of purified samples of Gandhaka, Haratala, Manahshila, intermediate products Samaguna kajjali, KH and KS, final products TS1, TS2 and SS are less than 1%w/w. This may be inferred as Bhavana given to KH1, KH2 and KS has insignificant influence on moisture content of final product. Hence it may be inferred as having the least moisture content and very rare chances of bacterial and fungal growth, i.e., the drug was having least hygroscopic nature with less chances of

contamination and expected shelf life of the product may be more.

Solubility test conducted in 14 different media on TS and SS suggested increased solubility in TS and SS compare to its respective kajjali in all media except petroleum ether and ether. The increased solubility might indicate better absorption and assimilation of final products to respective kajjali. The solubility of TS is relatively less than SS in all media. Solubility in honey confirms the use of honey as a best anupana for TS & SS. KH, KS, TS1, TS2 and SS1 samples were tested for contamination of TBC-total bacterial count (CFU/ gm), total fungal count (CFU/ gm), E.Coli, salmonella, P.aeruginosa and S.aureus. The microbial profile was conducted by inoculating the sample to agar media. If there were microbes in samples they proliferate in colonies and each colony was counted as one microbe and so the unit was given as 'Colonies For Unit' (CFU). Permissible TBC and total fungal counts were 10^5CFU/ gm and 100 CFU/ gm respectively. All the samples were having count below the normal limits for bacteria and fungus. The presence of minimum amount of microbes in samples might be due to atmospheric exposure. Absences of bacteria like E.Coli, salmonella, P.aeruginosa and S.aureus indicates that medicine prepared will not induce any infection. This may be inferred as hygienic methods of preparation; hence medicine is safe for consumption in regards with microbial contamination.

Quantitative analysis like estimation of total mercury is less in KH1 and KS when compared to TS and SS. This may be due decrease in concentration of sulphur due to the evaporation of free sulphur and few mercury particles while heating in an open air. TS and SS had mercury of about 61.02-59.11%. This mercury might have bonded with sulphur and arsenic (i.e., mercuric sulphide with arsenic). Quantity of free sulphur was comparatively more in KH1 and KS than TS and SS, traces in final products denotes the quantum of heat given to these products, as sulphur starts vaporizing and escaping around 120^{o}C and above, rest of sulphur gets bonded with mercury. Total sulphur content chronologically decreases in the final products which may signify sulphur in bonded form after escaping of free sulphur due to the quantum of heat given to these products. Quantity of arsenic was comparatively more in KH1 and KS than in TS and SS. There was reduction in concentrations of arsenic in final products compared to respective Kajjali. This might suggest evaporation of arsenic also during this process. Odour of arsenic during stage of fumes was more in SS than TS in all the trails. Quantitative analysis indicates less percentage of arsenic in SS when compared to TS. Both of them might suggest loss of more amount of arsenic in SS compared to that of TS.

CONCLUSION

TS and SS are Sagandha, Sagni, Kantastha Kupipakwa rasayanas. Kajjali is transformed to TS and SS with Agni samskara, thus altering the physico-chemical properties from that of Kajjali. During pharmaceutical work accumulation of Gandhaka near the neck of Kachakupi, prolonged stage of fumes and strong typical odour of arsenic, fuel consumption, manual labour and total duration of preparation of all samples of SS was comparatively more than respective samples of TS. So preparation of TS was easy and safe compared to SS. Microbial contamination showed the absence of infectious species and also other bacteria, yeast and moulds in minimal amounts. Thus Kajjali and respective Sindura are hygienic and are safe as they will not induce any infections. Quantitative analysis of ingredients of TS and SS were similar. This might suggest difference in lattice structure though chemically same, like cinnabar and Kajjali are HgS, but crystal lattice structure varies, leading to change in colour and properties. Relative clinical efficacy of TS and SS is a point of research. If the activity of TS is similar to SS, TS may be used as substitute to SS, since Haratala is relatively less toxic and market availability of Manashila is reducing in concern to social security.

ACKNOWLEDGEMENT: None

CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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