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PHYSICOCHEMICAL CHARACTERIZATION OF *DWIGUNA BALIJARITA* RASASINDURA (MERCURY SULPHIDE) PREPARED BY TWO METHODS

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Keywords:

Kupipakwa, XRD, Sulphur, *Jarana*, *Rasayana*

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ABSTRACT: Introduction: Rasashastra (Iatrochemistry) transforms raw minerals into therapeutically potent formulations such as *Rasasindura* (mercury sulphide). *Gandhaka Jarana* (sulphur digestion) is the key pharmaceutical process governing its formation and quality. **Objectives:** This study compares the pharmaceutical and physicochemical effects of Single-Stage versus Two-Stage (Sequential) *Gandhaka Jarana* in the preparation of *Dwiguna Rasasindura*. **Methods:** *Dwiguna Rasasindura* was prepared from purified mercury and Sulphur using the two pharmaceutical methods in which Single-Stage *Gandhaka Jarana* used *Kajjali* (black sulfide of mercury) was prepared with double the quantity of sulphur, and the Two-Stage (Sequential) *Gandhaka Jarana* involved the preparation of *Samaguna Rasasindura* followed by additional sulphur. Both preparations were processed by the *Kupipakwa* method under controlled heating conditions. Five distinct samples comprising *Samaguna Rasasindura* (SR), *Single-Stage Dwiguna Rasasindura* (SDR), *Two-Stage Dwiguna Rasasindura* (TDR), and their corresponding *Kajjali* intermediates (KJ-SDR and KJ-TDR), were analyzed using X-ray Diffraction (XRD) for crystallographic characterization and X-ray Fluorescence (XRF) for elemental analysis. **Result:** XRD analysis confirmed that all pharmaceutical methods resulted in the formation of crystalline cinnabar (HgS), although the *Kajjali* intermediates exhibited lower crystallinity. XRF analysis demonstrated mercury and sulphur as the principal elements in all preparations, with minor differences in elemental composition. Overall, the findings indicate that irrespective of the *Gandhaka Jarana* method employed, successful conversion of the formulation into crystalline *Rasasindura* (HgS) was achieved. **Conclusion:** The analytical findings alone cannot establish therapeutic equivalence, and further pharmacological and clinical studies are required to evaluate their biological and therapeutic properties.

INTRODUCTION: Ayurveda employs medicinal substances derived from plant, animal, and mineral sources¹.

Rasashastra (Iatrochemistry), the branch of Ayurveda dealing with mineral and metal-based formulations, involves specialized pharmaceutical procedures to process raw materials into formulations intended for therapeutic use. Among these, mercury occupies a central position and is subjected to procedures such as *Shodhana* (purification)², *Murchana* (potentiation)³, and *Jarana* (digestion/incorporation)⁴ to modify its

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physicochemical properties before incorporation into herbo-mineral formulations. These pharmaceutical processes are considered essential because crude mercury is not used directly for therapeutic purposes due to its known toxicity⁵.

Gandhaka Jarana (sulphur digestion) is one of the most important pharmaceutical procedures in the preparation of mercurial formulations. Classical texts emphasize that even properly purified mercury should not be administered therapeutically without undergoing adequate *Gandhaka Jarana*⁶. It is stated that mercury processed with sulphur acquires enhanced stability, therapeutic potency, and disease-curing capability, whereas inadequately processed mercury may produce undesirable effects⁷. During this process, mercury interacts with sulphur, leading to physicochemical transformations that contribute to the formation of mercury sulphide (HgS). *Rasasindura*, prepared through the *Kupipakwa Rasayana* process, is one of the most widely used mercurial formulations described in *Rasashastra*⁸. Classical texts describe several varieties of *Rasasindura* based on the quantity of sulphur incorporated, including *Samaguna*, *Dwiguna*, *Triguna*, *Chaturguna*, and *Shadaguna Rasasindura*, with onefold threefold, fourfold, and sixfold quantities of sulphur, respectively¹¹, indicating the pharmaceutical importance of sulphur proportion during preparation. Modern analytical techniques facilitate the scientific evaluation and quality assessment of traditional Ayurvedic formulations. X-ray Diffraction (XRD) enables characterization of crystalline phases, while X-ray Fluorescence (XRF) provides quantitative elemental analysis, thereby supporting the standardization and comparative evaluation of mineral-based formulations. Although classical texts describe different approaches for preparing *Dwiguna Rasasindura*, comparative analytical studies evaluating the influence of Single-Stage and Two-Stage (Sequential) *Gandhaka Jarana* on the physicochemical characteristics of the final product remain limited. In the Single-Stage method, the entire quantity of sulphur is incorporated during *Kajjali* preparation, whereas the Two-Stage (Sequential) method involves the preparation of *Samaguna Rasasindura* followed by incorporation of an additional equal quantity of sulphur and a

second *Kupipakwa* process. Whether these pharmaceutical approaches produce differences in the crystallographic and elemental characteristics of the final formulation has not been adequately investigated. Therefore, the present study was undertaken to prepare *Dwiguna Rasasindura* by Single-Stage and Two-Stage (Sequential) *Gandhaka Jarana*, document the pharmaceutical observations during preparation, and compare the resulting *Rasasindura* and their corresponding *Kajjali* intermediates using X-ray Diffraction (XRD) and X-ray Fluorescence (XRF). The study aimed to evaluate the influence of the two pharmaceutical approaches on the crystallographic and elemental characteristics of *Dwiguna Rasasindura*.

MATERIALS AND METHODS:

Procurement of Raw Material: Purified mercury (*Parada*), purified sulphur (*Gandhaka*), and fresh *Vatankura* (*Ficus benghalensis* Linn.) required for the preparation of *Rasasindura* were procured from the pharmaceutical laboratory of the Upgraded Department of *Rasashastra* and *Bhaishajya Kalpana*, Government Ayurved College, Vadodara, Gujarat, India. The raw materials were authenticated according to the standards of the Ayurvedic Pharmacopoeia of India before use.

Shodhana of Parada: *Parada* was purified according to the procedure described in *Rasatangini*¹². *Ashuddha Parada* (unpurified mercury) (500 g) was triturated with an equal quantity of *Sudha Churna* (lime powder) (500 g) in a *Khalva Yantra* (iron mortar) for three days, followed by washing with hot water to separate the mercury, which was then filtered through a double-folded cotton cloth. The collected *Parada* (452 g) was subsequently triturated with an equal quantity of *Nistusha Rasana* (*Allium sativum* Linn.) (452 g) and half its quantity of *Saindhava Lavana* (rock salt) (226 g) until a blackish *Kalka* (paste) was formed. This *Kalka* was washed with warm water, and the purified *Parada* was carefully collected, filtered through a double-folded cotton cloth, weighed, labelled, and stored in a glass container.

Shodhana of Gandhaka: *Gandhaka* was purified according to the procedure described in *Rasatangini*¹³. *Ashuddha Gandhaka* (unpurified sulphur) (1 kg)

was powdered using an iron mortar and pestle. *Godugdha* (cow milk) (6 L) was poured into a stainless-steel vessel, the mouth of which was covered with a cotton cloth smeared with *Goghrita* (cow ghee) (100 g) and securely tied. *Ashuddha Gandhaka* was melted in *Goghrita* over mild heat and carefully poured into the vessel containing *Godugdha*. The solidified *Gandhaka* was collected, washed with warm water, and the process was repeated three times in total. Finally, the purified *Gandhaka* was dried, weighed, and stored in a labelled airtight container for further pharmaceutical procedures.

Preparation of Dwiguna Rasasindura: All three variations of batches of *Rasasindura* and its *Kajjali* intermediates were prepared in the pharmaceutical laboratory of the Upgraded Department of Rasashastra and Bhaishajya Kalpana, Government Ayurved College, Vadodara, Gujarat, India.

Dwiguna Rasasindura prepared by Single-Stage Gandhaka Jarana: *Kajjali* was prepared by triturating *Shuddha Parada* (66 g) with *Shuddha Gandhaka* (132 g) in an iron mortar until *Nischandratva* (absence of metallic lustre) was attained. Trituration was continued for approximately 36 hours. The *Kajjali* was then levigated with freshly prepared *Vatankura Svarasa*

(60 mL) for about 8 hours until a homogeneous paste was obtained. The resulting paste was subsequently dried and powdered

The dried *Kajjali* was filled up to one-third of a seven-layered mud-smeared *Kupi* (glass bottle). The bottle was prepared by applying seven successive layers of cloth coated with Fuller's earth (*Kapadmitti*), allowing each layer to dry before applying the next. The *Kupi* was placed in a *Valuka Yantra* with its neck exposed above the sand level and heated according to the classical *Kupipakwa* procedure under continuous pyrometric monitoring.

The heating schedule consisted of *Mridu Agni* (mild heat) (room temperature to 150 °C for 1 h and 150 °C to 250 °C for 5 h), *Madhyama Agni* (moderate heat) (250 °C to 300 °C for 2 h, 300 °C to 350 °C for 3 h, 350 °C to 400 °C for 4 h, and 400 °C to 450 °C for 5 h), and *Tivra Agni* (intense heat) (450 °C to 650 °C for 8 h and 650 °C to 700 °C for 2 h). Endpoint observations, including disappearance of the flame, complete cessation of sulphur fumes, development of a red-colored base of the *Kupi*, and a positive copper coin test, were recorded before corking. The apparatus was then allowed to cool naturally. The final *Rasasindura* was collected after self-cooling **Fig. 1 Table 2**.



FIG. 1: STANDARD OPERATIVE PROCEDURE FOR DWIGUNA RASASINDOORA PREPARED BY SINGLE-STAGE GANDHAKA JARANA

Dwiguna Rasasindura prepared by Two-Stage (Sequential) Gandhaka Jarana:

Stage 1: Preparation of Samaguna Rasasindura:

Kajjali was prepared by triturating purified mercury 100 g and purified sulphur 100 g until *Nischandratva* was attained. Trituration was continued for approximately 30 hours, followed by levigation with 80 mL of *Vatankura Svarasa*. After drying, the *Kajjali* was processed by the *Kupipakwa* method using the same *Kupi* preparation, heating schedule, and endpoint observations described above to obtain *Samaguna Rasasindura*.

Stage 2: Preparation of Dwiguna Rasasindura:

The prepared *Samaguna Rasasindura* (63 g) was triturated with purified sulphur (100 g) until *Nischandratva* was achieved. The mixture was levigated with *Vatankura Svarasa* (80mL, dried under 43 h, filled into a seven-layered mud-smear *Kupi*, and subjected to a second *Kupipakwa* process following the same heating schedule and endpoint observations as described above. The final *Dwiguna Rasasindura* was collected after natural cooling **Fig. 2**.



FIG. 2: STANDARD OPERATIVE PROCEDURE FOR DWIGUNA RASASINDURA PREPARED BY TWO-STAGE (SEQUENTIAL) GANDHAKA JARANA

Physicochemical Characterization: Five samples were subjected to physicochemical characterization:

Sample 1: *Samaguna Rasasindura* (SR)

Sample 2: *Dwiguna Rasasindura* prepared by Single-Stage *Gandhaka Jarana* (SDR)

Sample 3: *Dwiguna Rasasindura* prepared by Two-Stage (Sequential) *Gandhaka Jarana* (TDR)

Sample 4: *Kajjali* prepared for Single-Stage *Dwiguna Rasasindura* (KJ-SDR)

Sample 5: *Kajjali* prepared for Two-Stage *Dwiguna Rasasindura* (KJ-TDR)

Crystallographic identification by X-ray Diffraction (XRD):

Crystallographic characterization of all samples was carried out using X-ray Diffraction (XRD) on an Empyrean X-ray Diffractometer. The analysis was performed under the following conditions: Cu K α radiation source, wavelength 1.5406 Å, voltage 40 kV, current 30 mA, scanning range 5.03°–79.96° (2 θ), step size 0.026°, and continuous scan mode with a scan step time of 125.715 s per step. Phase

identification was performed using the PDF-2 database of the International Centre for Diffraction Data (ICDD), while crystallographic information was verified using the American Mineralogist Crystal Structure Database (AMCSD).

Quantitative Elemental Determination by X-ray Fluorescence (XRF): Quantitative elemental analysis was carried out using X-ray Fluorescence (XRF) on an EDXRF spectrometer. Samples were prepared by finely grinding the powdered samples to obtain a homogeneous particle size and loading them into XRF sample cups fitted with polypropylene film, ensuring a flat and uniform

sample surface for analysis. Instrumental conditions included energy-dispersive X-ray fluorescence operated under vacuum with automatic selection of excitation conditions using multiple secondary targets, an X-ray tube voltage of 50 kV, tube current adjusted automatically by the instrument, and optimized counting time for quantitative elemental analysis.

The concentrations of mercury, sulphur, and other inorganic elements were determined and expressed as percentage composition for comparative evaluation of all samples.

RESULTS:

TABLE 1: PHARMACEUTICAL OBSERVATIONS AND PERCENTAGE YIELD OF RASASINDURA PREPARED BY DIFFERENT METHODS

Parameter	SR	SDR	TDR
Initial <i>Kajjali</i> weight (g)	200	200	163
Final <i>Rasasindura</i> obtained (g)	69	85	48
Material loss (g)	131	115	115
Colour	Crimson red	Crimson red	Crimson red
Yield (%) = Final <i>Rasasindura</i> weight / Initial <i>Kajjali</i> weight × 100	34.5	42.5	29.44

TABLE 2: COMPARATIVE TEMPERATURE, TIME, AND PHARMACEUTICAL OBSERVATIONS DURING THE PREPARATION OF SAMAGUNA RASASINDURA (SR), SINGLE-STAGE DWIGUNA RASASINDURA (SDR), AND TWO-STAGE DWIGUNA RASASINDURA (TDR)

Observations	SR		SDR		TDR	
	Time (h: min)	Temperature (°C)	Time (h: min)	Temperature (°C)	Time (h:min)	Temperature (°C)
Heating started	00:00	27	00:00	21	00:00	33
Appearance of white fumes	00:30	122	00:30	120	00:30	83
Smell of sulphur perceived	01:30	156	02:00	163	02:00	120
Appearance of sulphur fumes	02:00	175	02:15	175	02:15	160
Melting of <i>Kajjali</i> started	03:30	220	05:00	206	05:00	283
Complete melting of <i>Kajjali</i>	06:00	410	06:30	442	06:30	452
Appearance of Blue flame	15:15	480	16:00	564	17:00	540
Flame disappeared	19:10	524	20:10	576	20:40	502
Appearance of Red coloured base of <i>Kupi</i>	19:30	550	21:50	580	21:30	540
Corking	20:00	600	22:00	590	22:00	590
Heating stopped	30:00	710	30:00	702	30:00	708

TABLE 3: HEATING PROFILE AND CORRESPONDING PHARMACEUTICAL OBJECTIVES DURING THE PREPARATION OF DIFFERENT RASASINDURA SAMPLES

Heating phase	SR		SDR		Objectives
	Hrs	Time	Temperature	TDR	
<i>Mridu Agni</i>	1	00:00 to 01:00	Room temperature to 150 °C for 1 h		To melt the <i>Kajjali</i> and initiate uniform heating.
	5	01:00 to 06:00	150 °C to 250 °C for 5 hr		
<i>Madhyama Agni</i>	2	06:00 to 08:00	250 °C to 300 °C for 2 hr		To complete the evaporation of excess sulphur and obtain a clear red molten base at the bottom of the <i>Kupi</i> .
	3	08:00 to 11:00	300 °C to 350 °C for 3 h		
	4	11:00 to 15:00	350 °C to 400 °C for 4h		
<i>Tivra Agni</i>	5	15:00 to 20:00	400 °C to 450 °C for 5h		To complete sublimation and obtain a well-deposited, crystalline <i>Rasasindura</i> in the neck of the <i>Kupi</i>
	8	20:00 to 28:00	450 °C to 650 °C for 8 h		
	2	28:00 to 30:00	650 °C to 700 °C for 2 h		

TABLE 5: DETAILS OF XRD REPORTS OF DWIGUNA RASASINDURA PREPARED BY SINGLE-STAGE GANDHAKA JARANA (SDR)

Compound	Cinnabar: Mercury Sulfide	Sulphur	Rosickyite
Composition	Hg S	S	S
Crystal shape	Hexagonal axis	Ortho rhombic	Mono clinic
2 theta	30.743	27.562	29.913
	36.220	33.481	33.979
	50.936	38.848	46.427
	3.3745	3.7551	3.4659
D spacing	2.8776	3.1054	3.0612
	2.0802	2.6898	2.2694
	15.6	40	37.9
Intensity %	35.3	19.2	16.7
	20.4	19.8	19.8

Sample 3: Dwiguna Rasasindura prepared by Two-Stage Gandhaka Jarana (TDR)

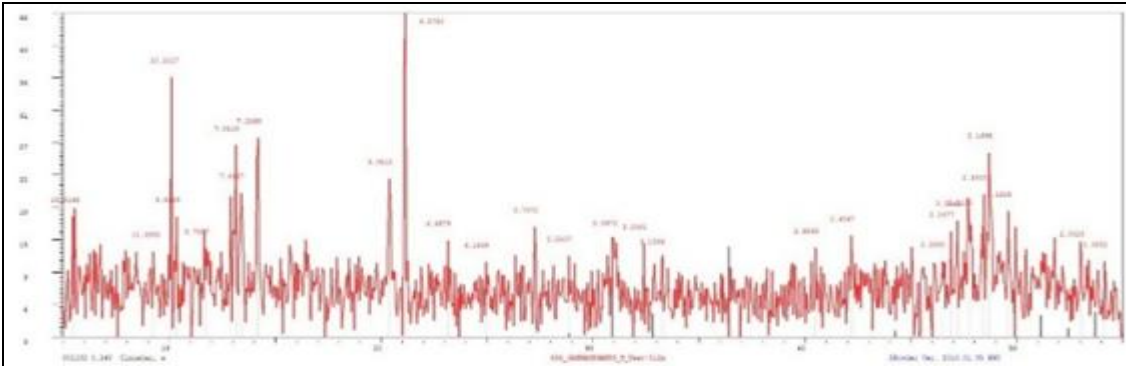


FIG. 5: DETAILS OF XRD REPORTS OF DWIGUNA RASASINDURA PREPARED BY TWO-STAGE GANDHAKA JARANA (TDR)

TABLE 6: DETAILS OF XRD REPORTS OF DWIGUNA RASASINDURA PREPARED BY TWO-STAGE GANDHAKA JARANA (TDR)

Compound	Cinnabar: Mercury Sulfide	Sulphur
Composition	Hg S	S
Crystal shape	Hexagonal axis	Orthorhombic
2 theta	30.936	27.562
	32.900	33.481
	51.157	38.848
	3.3539	3.7551
D spacing	3.1587	3.1054
	2.0718	2.6898
	30.7	40
Intensity %	22.6	19.2
	23.3	19.8

Sample 4: Kajjali of Dwiguna Rasasindura prepared by Single-Stage Gandhaka Jarana

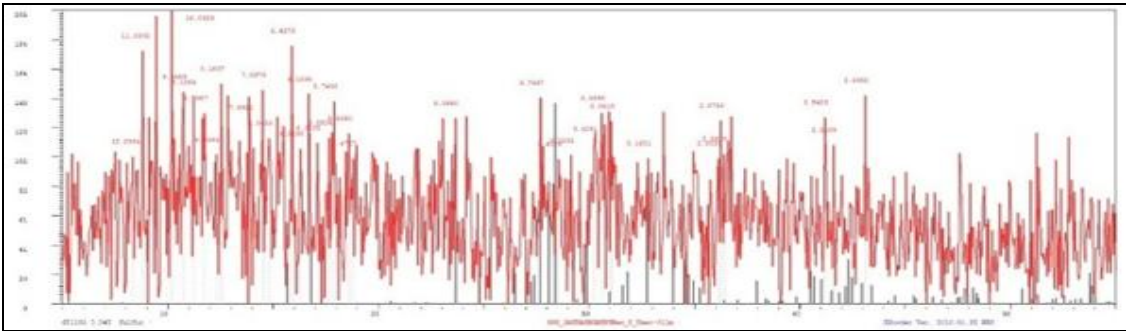


FIG. 6: XRD SPECTRUM OF KAJJALI OF DWIGUNA RASASINDURA PREPARED BY SINGLE-STAGE GANDHAKA JARANA

TABLE 7: DETAILS OF XRD REPORTS OF DWIGUNA RASASINDURA PREPARED BY SINGLE-STAGE GANDHAKA JARANA (KJ-SDR)

Compound	Cinnabar: Mercury Sulfide	Sulphur
Composition	Hg S	S
Crystal shape	Cubic	Monoclinic
2 theta	30.383	23.661
	35.279	28.032
	50.659	32.707
	3.4135	4.3629
D spacing	2.9518	3.6933
	2.0908	3.1769
	35.5	63.3
Intensity %	33.1	38.0
	17.7	38.6

Sample 5: Kajjali of Dwiguna Rasasindura prepared by Two-Stage Gandhaka Jarana (KJ-TDR)

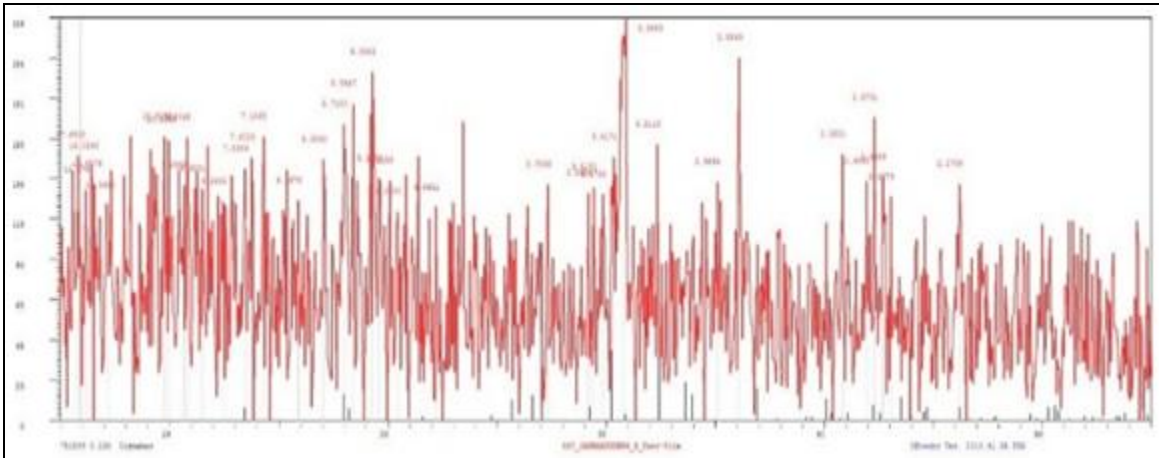


FIG. 7: XRD SPECTRUM OF KAJJALI OF DWIGUNA RASASINDURA PREPARED BY TWO-STAGE GANDHAKA JARANA

TABLE 8: DETAILS OF XRD REPORTS OF DWIGUNA RASASINDURA PREPARED BY TWO-STAGE GANDHAKA JARANA (KJ-TDR)

Compound	Cinnabar: Mercury Sulfide	Syn Alpha Sulfur
Composition	Hg S	S8
Crystal shape	Hexagonal axis	Orthorhombic
2 theta	30.743	13.426
	36.220	17.908
	50.963	26.593
	3.3745	7.6517
D spacing	2.8776	5.7472
	2.0791	3.8892
	93.5	62.4
Intensity %	45.4	41.0
	22.5	41.6

Quantitative Elemental Determination (XRF): XRF analysis was carried out to determine the elemental composition of the prepared *Rasasindura* samples. Mercury (Hg) and sulfur (S) were found to be the predominant elements in all samples. *Samaguna Rasasindura* contained 76.05% Hg and 20.50% S. *Dwiguna Rasasindura* prepared by Single-Stage *Gandhaka Jarana* showed 67.99% Hg and 19.47% S, along with a detectable amount of

iron (1.136%). *Dwiguna Rasasindura* prepared by Two-Stage *Gandhaka Jarana* contained 76.02% Hg and 20.21% S. Trace quantities of elements such as Si, P, Ti, Ni, Cu, As, Rb, Zr, Nb, Mo, Sn, Sb, Pb, and Th were detected in varying amounts across all samples. The elemental profiles of *Samaguna Rasasindura* and *Dwiguna Rasasindura* prepared by two-Stage *Gandhaka Jarana* were found to be comparable, whereas *Dwiguna*

Rasasindura prepared by Single-Stage *Gandhaka Jarana* showed relatively lower mercury content and the presence of iron.

DISCUSSION: The pharmaceutical observations demonstrated that all three preparations successfully yielded crimson-red *Rasasindura*, indicating successful completion of the *Kupipakwa* process and formation of mercuric sulphide (HgS). Although identical heating schedules were employed, considerable variation was observed in the percentage yield. Single-Stage *Dwiguna Rasasindura* (42.5%) produced the highest yield, followed by *Samaguna Rasasindura* (34.5%), whereas Two-Stage *Dwiguna Rasasindura* showed the lowest yield (29.44%). The factor contributing to the lower recovery in TDR is the use of previously prepared *Samaguna Rasasindura* rather than fresh *Kajjali*. Since a portion of mercury has already been converted into crystalline HgS after the first heating cycle, further sulphur incorporation during the second stage primarily affects the surface of existing HgS particles rather than initiating an entirely new mercury-sulphur reaction. Therefore, yield alone should not be considered an indicator of pharmaceutical quality, as greater recovery does not necessarily correspond to greater chemical conversion or improved crystallinity.

The initial white fumes were probably due to the evaporation of residual moisture from *Kajjali* and *Vatankura Svarasa* during heating. The subsequent appearance of sulphur odour and fumes corresponded to the melting and volatilization of sulphur as the temperature increased. *Kajjali* melting occurred later in SDR and TDR than in SR, probably because the higher sulphur content increased the viscosity of the molten mass and delayed the formation of a homogeneous liquid phase. The appearance of the blue flame indicated combustion of sulphur vapours, while its delayed occurrence in SDR may be attributed to the larger quantity of sulphur available for volatilization. Disappearance of the flame suggested depletion of free sulphur vapours, and the subsequent development of a red molten base indicated predominant formation of mercury sulphide (HgS). XRF analysis demonstrated that mercury and sulphur were the predominant elements in all three *Rasasindura* preparations, while XRD analysis confirmed the presence of crystalline mercury

sulphide (HgS), indicating successful preparation of *Rasasindura* by both pharmaceutical methods. *Samaguna Rasasindura* (SR) and Two-Stage *Dwiguna Rasasindura* (TDR) exhibited comparable mercury concentrations (76.05% and 76.02%, respectively), suggesting that sequential *Gandhaka Jarana* produced an elemental composition similar to that of classical *Samaguna Rasasindura*. In contrast, Single-Stage *Dwiguna Rasasindura* (SDR) showed a relatively lower mercury content (67.99%), which may be attributed to the higher proportion of sulphur incorporated during the initial *Kajjali* preparation. Since XRF expresses elemental concentrations as normalized percentages of the total sample mass, a relatively higher sulphur content can decrease the apparent percentage of mercury without necessarily indicating mercury loss. Overall, the analytical findings indicate that all three preparations were successfully converted into HgS, with only minor variations in elemental composition arising from differences in the pharmaceutical sequence of sulphur incorporation.

CONCLUSION: The present physicochemical and crystallographic findings confirm the formation of cinnabar-HgS in all three preparations, but these analytical similarities alone cannot establish therapeutic equivalence. Further pharmacological and well-designed clinical studies are required to evaluate whether differences in *Jarana* methodology influence the therapeutic efficacy and clinical outcomes of *Rasasindura*.

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