Pharmaceutico-Analytical Standardization and In-vitro Anti-lung Cancer Activity of *ShadgunabalijaritaRasa Sindoor*Against A-549 Human Lung Adenocarcinoma Cell Line.

ABSTRACT:

Background:

Lung cancer is one of the most common causes of cancer associated mortality worldwide and limits of conventional therapy has resulted in interest in traditional medicinal formulations. *ShadgunabalijaritaRasa Sindoor*is one of the classical *KupipakwaRasayana*preparations used traditionally for different diseases but its pharmacological standardisation and anticancer potential needs scientific substantiation.

Objective:

1. To standardise *ShadgunabalijaritaRasa Sindoor*in classical ayurvedic procedures.
2. To study its pharmaceutico-analytical features
3. To evaluate its in vitro cytotoxic effect on A-549 human lung cancer cell line.

Materials and Methods:

The formulation was manufactured by classical processes of *Rasashastra*after due purification and pharmaceutical processing. The final product was investigated for pharmaceutico-analytical parameters including instrumental characterisation. Cytotoxic activity against A-549 cells was tested by MTT assay. Cell viability and IC-50 values were established.

Results:

The standardised preparation was found to be a bright Red *Rasa Sindoor* with good pharmaceutic properties. XRD verified the existence of crystalline mercury sulphide (HgS) in the cinnabar phase while XRF showed mercury and sulphur as the primary ingredients. The formulation showed concentration dependent cytotoxicity against A-549 cells with IC50 value of 76.41 µg/mL along with typical morphological alterations.

Conclusion:

The *ShadgunabalijaritaRasa Sindoor*was effectively standardised and analytically characterised. The formulation showed potential in-vitro cytotoxic

efficacy against A-549 lung cancer cells and urge additional mechanistic, pre-clinical and clinical investigations.

Keywords:*ShadgunabalijaritaRasa Sindoor*,*Rasashastra*,KupipakwaRasayana, Pharmaceutical standardization,XRD,XRF, A-549 cell line, MTT assay, Cytotoxicity.

1.Introduction.

Despite considerable breakthroughs in early detection, targeted therapy and immunotherapy, lung cancer remains one of the major causes of death from cancer worldwide. The prognosis of advanced disease is poor because to late diagnosis, tumour heterogeneity, therapeutic resistance and side effects of the treatment. These constraints have generated interest in the identification of safer and more effective therapeutic agents including those originating from traditional systems of care.(1)

Natural products have played a major role in the discovery of anticancer drugs and many essential anticancer drugs in clinical use are of plant origin. Ayurveda, the traditional system of Indian medicine, mentions a large number of herbal and herbo-mineral preparations with therapeutic potential. In its specialised branches, *Rasashastra*uses pharmaceutical techniques like *Shodhana*, *Jarana*, *Bhavana* and controlled heating to convert raw ingredients into therapeutically acceptable formulations. It is necessary that these preparations are scientifically standardised, thus assuring their quality, reproducibility and safety.(2)

ShadgunabalijaritaRasa Sindoor(3)is a typical *KupipakwaRasayana*(4)made from refined mercury and sulphur by repeated pharmaceutical processing. Such pharmacological changes have been credited with improved therapeutic capabilities in the classical literature, but systematic scientific evidence on its pharmaceutical standardisation, analytical features and biological activity is scarce. Modern analytical techniques such as X-ray diffraction (XRD) and X-ray fluorescence (XRF) and in-vitro biological assays are useful instruments for the assessment of the physicochemical features and biological potential of these formulations.(5)(6)

Hence, present study was undertaken to prepare *ShadgunabalijaritaRasa Sindoor*as per classical Ayurvedic pharmaceutical procedures, perform pharmaceutico-analytical standardisation using modern analytical techniques and to evaluate its in-vitro cytotoxic activity against human lung adenocarcinoma (A-549) cell line.(7) The present investigation is aimed to

create scientific data in support of the quality, reproducibility and preliminary anticancer potential of this historical *Rasashastra*formulation.

2. Materials and Methods

2.1 Study Design

The present experimental work was undertaken to investigate *ShadgunabalijaritaRasa Sindoor*by integrated pharmaco-analytical and biological studies.

The study was carried out in three sequential phases:

(i) pharmaceutical preparation of *ShadgunabalijaritaRasa Sindoor*as per the classical principles of *Rasashastra*,

(ii) pharmaceutico-analytical characterisation of the prepared formulation by classical and modern analytical techniques and

(iii) evaluation of in-vitro cytotoxic activity against the human lung adenocarcinoma (A-549) cell line using MTT assay(8). The complete workflow of the investigation is presented in Figure 1.

2.2 Procurement and Authentication of Raw Materials

The pure mercury (*Shuddha Parada*)(9) and purified sulphur (*Shuddha Gandhaka*)(10) were obtained from the recognised sources and tested for their organoleptic characters before usage. The processing of pharmaceuticals like *Shodhana*, *Kajjali* preparation(11) and the other pharmaceutical procedures were undertaken as per the established protocols given in the classical *Rasashastra* literature.

Raw material collection: -

2.3 Pharmaceutical Preparation of *Shadgunabalijarita* Rasa Sindoor(12)

The preparation was done in the Department of *Rasashastra*and*BhaishajyaKalpana* by classical manner of *KupipakwaRasayana*.

Table 1. Pharmaceutical Processing of Raw Materials

Sr. No.	**Pharmaceutical Process**	**Classical Reference**	**Initial Weight (g)**	**Final Weight (g)**
1	*Shodhana*of *Parada*	*Parada Samhita*(13)	108	105
2	*Shodhana*of*Gandhaka*	*Rasatarangini*	614	602
3	Preparation of *Kajjali*	*Rasatarangini*	—	675
4	*Bhavana* of*Kajjali*(12)	—	675	685

Purified mercury and purified sulphur were triturated to give uniform lustreless black *Kajjali*. *ShadgunabalijaritaKajjali* was prepared by doing later *Jarana* with sulphur and *Bhavana* with *KumariSwarasa* for 6 cycles.

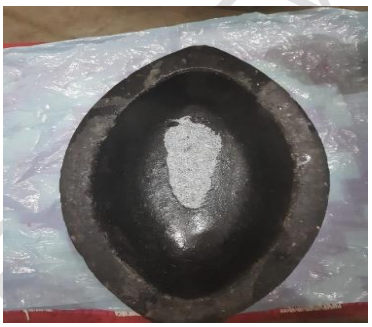
The prepared *Kajjali* was filled in seven layered Kacha *Kupi* and heated in *Valuka Yantra/* EMF under controlled *Mridu, Madhyama* and *Tivragni*. Classical pharmaceutical observations were made during the heating process such as colour changes, sulphur emissions, features of the flame and product deposition. The bright red crystalline *Rasa Sindoor* recovered at the neck ofthe bottle after self-cooling was pulverised and stored in sealed glass containers for further analytical and biological examination.

Purification of Parad: -



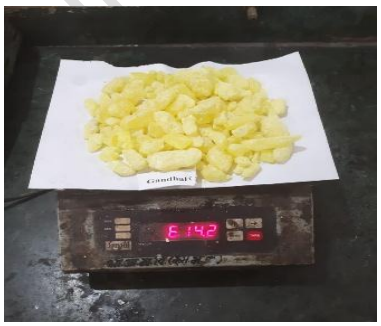




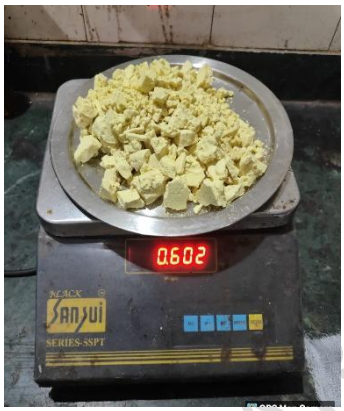




117

118 **Purification of Gandhak**







119 **Formation of ShadgunabalijaritaKajjali:-**



120 **Bhavana To Prepared Kajjali**



121

122

123 Table 2: Comparative Batch wise pharmaceutical preparation of
124 *ShadagunabalijaritaRasasindoor*

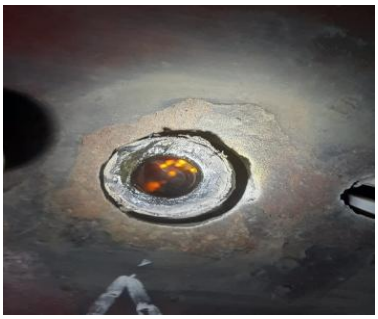
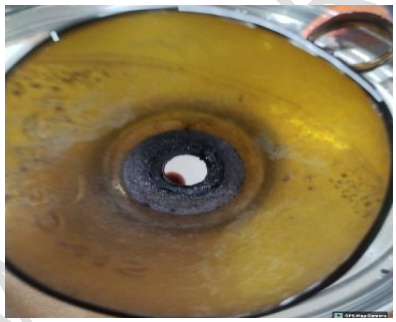
Time (hrs)	**Batch-I (Temp °C / Observation)**	**Batch-II (Temp °C / Observation)**	**Batch-III (Temp °C / Observation)**
0	50°C (Accel. 5) – Kupi started	50°C (Accel. 5) – EMF started	50°C (Accel. 5) – EMF started
2–3	110°C – Puffiness in *Kajjali*, *Gandhaka* smell	120°C – Puffiness of *Kajjali*	120°C – Puffiness of *Kajjali*
4	160°C – *Kajjali* melted, yellow fumes	160°C – Yellow fumes with smell	160°C – Yellow fumes with smell
6	200°C – Yellow fumes with smell	183°C – Dense yellow + white fumes	183°C – Dense yellow + white fumes
10	250°C – *Kajjali* started melting, dense yellow fumes	260°C – Dense yellow fumes, neck deposition	260°C – Dense yellow fumes, neck deposition
14	320°C – Dense yellow fumes, bottom not visible	323°C – Slight golden-orange base, fumes continue	323°C – Slight golden-orange base, fumes continue

16–18	360°C – Blue flame during Hot *ShalakaSanchalana*	350°C – Golden-red base visible	350°C – Golden-red base visible
20–22	387°C – *Kajjali* boiling, yellow fumes	390°C – Reddish-orange base with fumes	390°C – Reddish-orange base with fumes
24–26	410–425°C – Golden-red base, neck deposition	407°C – *Kajjali* boiling, dense fumes; 420°C – Neck deposition, red base	407°C – *Kajjali* boiling, dense fumes; 420°C – Neck deposition, red base
30	412°C – Blue flame after Hot *Shalaka*	420°C – Dry red base, blue flame	420°C – Dry red base, blue flame
34	430°C – Yellow fumes, blue flame continues	425°C – Yellow fumes, red-hot base, blue flame	425°C – Yellow fumes, red-hot base, blue flame
36	—	425°C – Yellow fumes, blue flame	425°C – Yellow fumes, blue flame
38	420°C – Fumes markedly reduced, neck deposition increased	425°C – No fumes, no blue flame after Hot *Shalaka*	425°C – No fumes, no blue flame after Hot *Shalaka*

40	460°C – Orange-red base, copper coin test negative	475°C – Copper coin test positive, corking, *Tivragni* started	475°C – Copper coin test positive, corking, *Tivragni* started
44	450°C – Golden-red base, no further changes	575°C – *Tivragni* continued	575°C – *Tivragni* continued
46	479°C – Copper coin test positive, corking, *Tivragni* started	625°C – *Tivragni* continued	625°C – Heating stopped (EMF switched off)
48	540°C – *Tivragni* continued	640°C – Heating stopped	—
50	610°C – *Tivragni* continued	—	—
51	660°C – EMF stopped	—	—

125 **Preparation of ShadgunabalijaritaRasasindoora:-**

		
		
		
1st Batch	**2nd Batch**	**3rd batch**

126

127

128 **2.4 Pharmaceutical Evaluation**

129 Critical observations were recorded throughout the*Kupipakwa* process to
130 monitor the pharmaceutical preparation. These observations included colour
131 changes, consistency, flame characteristics, evolution of fumes, corking stage,
132 conclusion of the reaction and ultimate yield and % recovery.

133 The prepared *ShadgunabalijaritaRasa Sindoor*was further assessed for its
134 satisfactory preparation with classical Ayurvedic standards.

2.5 Pharmaceutico-Analytical Characterization

The prepared *ShadgunabalijaritaRasa Sindoor*was evaluated pharmacutico-analytically for the establishment of quality and physicochemical features. Organoleptic features such as colour, odour, look and texture were noted. The physicochemical analysis was carried out according to the normal pharmacopoeial protocols. The constituent composition, crystalline phase and surface morphology were determined by instrumental characterisation using X-ray fluorescence (XRF), X-ray diffraction (XRD). All studies were performed under recognised laboratory methods(14).

2.6 In-vitro Cytotoxicity Assay

The cytotoxic effect of *ShadgunabalijaritaRasa Sindoor*was studied on human lung adenocarcinoma (A-549) cell line by MTT assay. Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with foetal bovine serum and antibiotics under standard culture conditions (37°C, 5% CO2). The test formulation was made at varied concentrations and incubated with A-549 cells in 96-well microplates for the indicated exposure time. Cell viability was assessed by MTT assay after treatment and the absorbance was detected using microplate reader. Cell viability was represented as a percentage of viable cells relative to the untreated control.(15)

2.7 Determination of IC_{50}

The percentage cell viability at varying concentration of test formulation was shown against dose response curves. The half-maximal inhibitory concentration (IC-50) was obtained using non-linear regression analysis.

2.8 Statistical Analysis

All experiments were performed in triplicate and findings are expressed as mean ± standard deviation (SD). Dose-response analysis and IC-50 determination were carried out using non-linear regression analysis. Statistical significance was accepted at p value <0.05

3. Results

3.1 Pharmaceutical Preparation of *ShadgunabalijaritaRasa Sindoor*

Table 1. Pharmaceutical Processing of Raw Materials

Sr. No.	Pharmaceutical Process	Classical Reference	Initial Weight (g)	Final Weight (g)	Percentage Change (%)
1	*Shodhana* of *Parada*	*Parada Samhita*	108	105	−2.7
2	*Shodhana*of*Gandhaka*	*Rasatarangini*	614	602	−1.9
3	Preparation of *Kajjali*	*Rasatarangini*	700	675	−3.6
4	Bhavana of *Kajjali*	*Rasatarangini*	675	685	+1.4

Pharmaceutical Observations

Shodhana of Parada

- The weight loss after purification was from 108 g to 105 g.
- Purified Parada had a better lustre and mobility.
- Visible foreign matter was removed.

Shodhana of Gandhaka

- Weight was reduced from 614 g to 602 g.

- Purified Gandhaka was bright yellow, supple and free from obvious contaminants.

Preparation of *Kajjali*

- *Kajjali* became a uniform coloured, fine, lusterless black powder.
- Classical quality checks showed Rekhapurnata and Nishchandratva acceptable.

Bhavana of *Kajjali*

- Weight increased from 675 g to 685 g due to uptake of the Bhavana medium.
- The fineness was increased and material was made more uniform.

Preparation of *Shadgunabalijarita* Rasa Sindoor

- Successfully prepared three batches.
- The time of heating was 48 to 51 hours.
- The final yield of *Rasa Sindoor* was in the range of 28 g to 30 g.
- The substance was a vivid red crystalline powder, as described in classical literature.

Table 2. Summary of Pharmaceutical Preparation of *ShadgunabalijaritaRasa Sindoor*

Parameter	**Observation**
Initial material	*ShadgunabalijaritaKajjali*
Pharmaceutical method	Classical *KupipakwaRasayana*
Heating schedule	*Mridu → Madhyama → Tivragni*

Number of batches	3
Heating duration	48–51 h
Final appearance	Bright red crystalline powder
Texture	Fine and smooth
Lustre	Characteristic bright lustre
Final yield	28–30 g per 200 g *Kajjali*
Batch reproducibility	Satisfactory

Results

The conventional *KupipakwaRasayana*process was successfully used to prepare *ShadgunabalijaritaRasa Sindoor*. Physicochemical changes were noticed sequentially during the pharmaceutical processing, suggesting the gradual transformation of *Kajjali* into the finished product. The original homogenous lustreless black *Kajjali* underwent characteristic changes during regulated heating. These changes included evolution of fumes, deposition of sublimed material in the neck of the Kacha *Kupi* and successful corking at the proper stage. The final product was obtained as a fine bright red crystalline powder deposited in the neck of the container after self-cooling, as in the ancient descriptions. The preparation was reproducible in all three batches with heating time of 48-51 h and had a constant yield of 28-30 g per 200 g *Kajjali*.

Table 3. Batch-wise Preparation of *Shadgunabalijarita* Rasa Sindoor

Reference:*Rasatarangini*, Chapter 6, Verses 162–167

Batch	Quantity of *Kajjali* Taken (g)	Heating Duration (Hours)	Final Yield (g)	Yield %
I	200	51	28	14
II	200	48	30	15
III	200	48	30	15

3.2 Organoleptic Evaluation

The last *ShadgunabalijaritaRasa Sindoor*demonstrated organoleptic features that were indicative of adequate pharmaceutical processing. Formulation was brilliant red fine crystalline powder with smooth texture and free from apparent foreign materials. No evidence of any disagreeable smell or dampness was discovered indicating acceptable stability and suitable storage conditions.(16)

Table 2. Organoleptic Characteristics

Parameter	Observation
Colour	Bright red
Appearance	Fine crystalline powder
Texture	Smooth
Odour	Odourless / characteristic
Moisture	Absent
Foreign matter	Not detected

3.3 Classical Quality Assessment

The developed formulation was found to meet the conventional quality requirements prescribed for *Rasa Sindoor*. The pharmaceutical procedure was completed and the formulation was suitable for further analytical and biological evaluation, as confirmed by correct colour development, successful *Kupipakwa* transformation and excellent product recovery, which are characteristic pharmaceutical observations.

3.4 Physicochemical Evaluation

Physicochemical assessment of the produced formulation showed good quality features. The measured analytical values, low moisture content and acceptable inorganic residual, suggest satisfactory pharmaceutical processing and uniformity of the batch.(17)

Table 3. Physicochemical Parameters

Parameter	Observation
Loss on drying	0.51% W/W
Total ash	0.23% W/W
Acid insoluble ash	0.14% W/W
Water soluble extractive	0.11% W/W
pH	Not Recorded

3.5 Instrumental Characterization

Further instrumental characterisation confirmed the identification and structural features of the produced formulation. XRD showed the characteristic diffraction peaks of crystalline mercuric sulphide (α-HgS). XRF analysis verified mercury and sulphur as the major elemental components, supporting the predicted composition of *Rasa Sindoor*.

Table 4. Summary of Instrumental Analysis

Investigation	Major Findings
XRD	Characteristic peaks of mercuric sulphide
XRF	Predominantly mercury and sulphur, consistent with mercuric sulphide (HgS)

3.6 In-vitro Cytotoxic Activity Against A-549 Cell Line

*ShadgunabalijaritaRasa Sindoor*displayed concentration dependant cytotoxic activity against A-549 human lung cancer cell line. With increasing dosage of the formulation cell viability was gradually reduced demonstrating dose-dependent suppression of cancer cell proliferation.

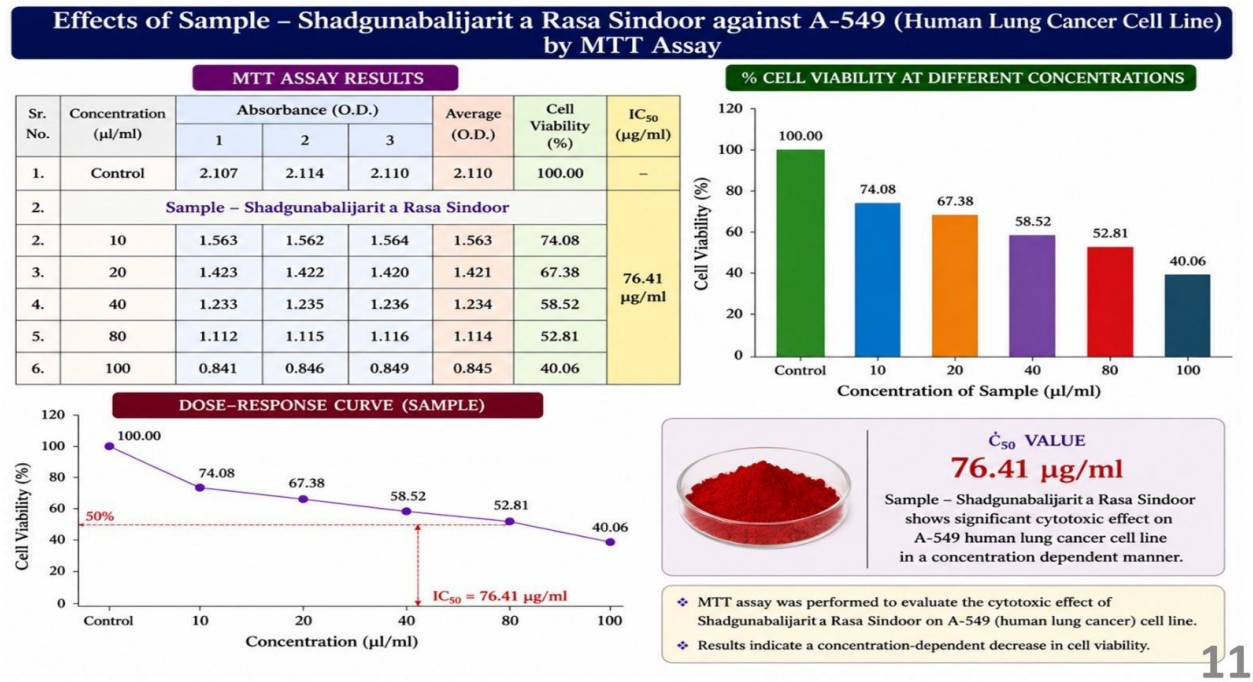
Microscopic studies showed morphological changes in treated cells including cell shrinkage and rounding, loss of cellular adherence and lower confluency compared to untreated control cells. These morphological changes also corroborated the cytotoxic effect of the mixture.

Table 5. Cytotoxic Activity Against A-549 Cell Line

Concentration	Cell Viability (%)	Absorbance average 3 batches (O.D)	Growth Inhibition (%)
Control	100	2.110	0
10	74.08	1.563	25.92
20	67.38	1.421	43.08
40	58.52	1.234	60.83
80	52.81	1.114	73.14
100	40.06	0.845	81.31

Figure 1.

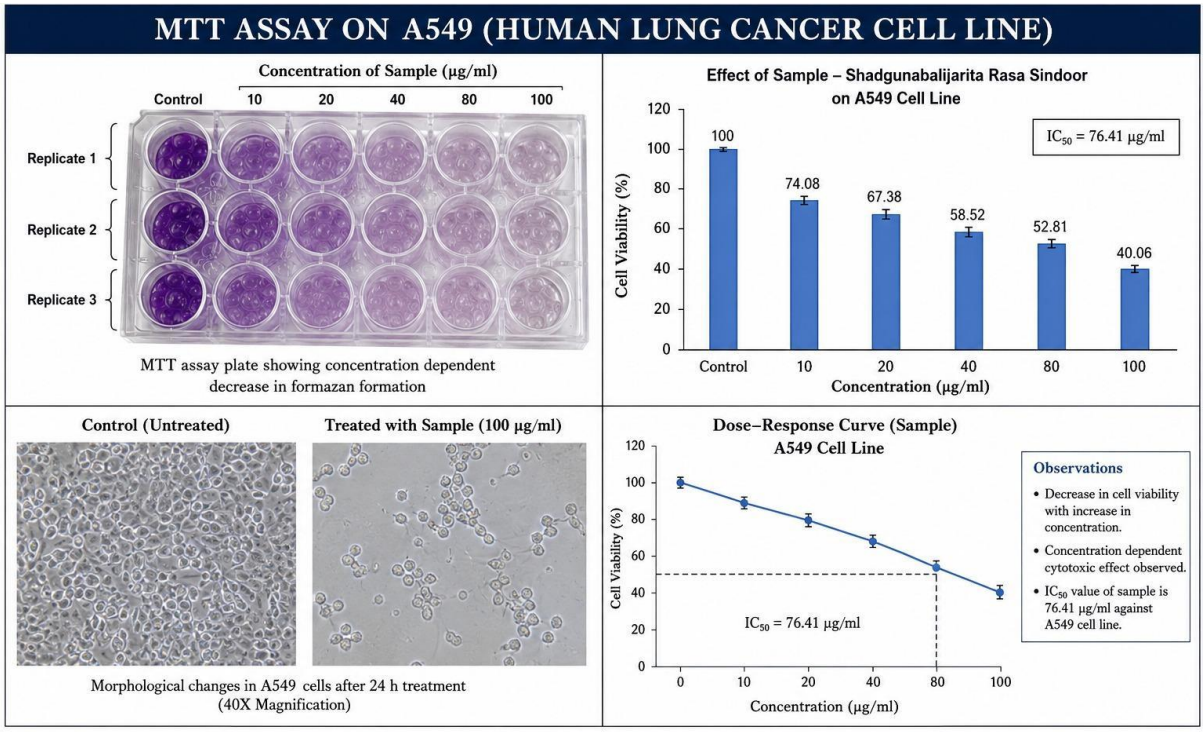
Dose-response curve showing concentration-dependent inhibition of A-549 cell viability by *Shadgunabalijarita* Rasa Sindoor.



Sr. No.	Concentration (µl/ml)	Absorbance (O.D.) 1	2	3	Average (O.D.)	Cell Viability (%)	IC_{50} (µg/ml)
1.	Control	2.107	2.114	2.110	2.110	100.00	–
2.	Sample – Shadgunabalijarit a Rasa Sindoor						76.41 µg/ml
2.	10	1.563	1.562	1.564	1.563	74.08	
3.	20	1.423	1.422	1.420	1.421	67.38	
4.	40	1.233	1.235	1.236	1.234	58.52	
5.	80	1.112	1.115	1.116	1.114	52.81	
6.	100	0.841	0.846	0.849	0.845	40.06	

Figure 2.

Representative microscopic images of untreated control and treated A-549 cells demonstrating concentration-dependent morphological alterations.



3.7 IC_{50} Determination

Dose response research indicated measurable cytotoxic activity of *ShadgunabalijaritaRasa Sindoor*against A-549 cells. The IC50 (the concentration that would inhibit 50% cell viability under the experimental conditions) was found to be 76.41 µg/mL. This finding verifies the in-vitro quantifiable antiproliferative effect of the formulation against A-549 cells.

3. Results Summary

The classical process of *KupipakwaRasayana*was successfully adopted for the preparation of *ShadgunabalijaritaRasa Sindoor*and it presented characteristic organoleptic traits with good physicochemical quality. XRD showed the crystalline form of α-HgS and XRF showed the presence of mercury and sulphur as the main elemental components. The formulation exhibited cytotoxicity against A-549 cell line in a concentration dependent

manner with an IC 50 of 76.41 µg/mL. This was further confirmed by the characteristic morphological alterations in the treated cells.

4. Discussion

Pharmaceutical Standardization of *Shadgunabalijarita* Rasa Sindoor

The successful preparation of *ShadgunabalijaritaRasa Sindoor*using classical *KupipakwaRasayana*techniques shows that the pharmaceutical approach chosen was reproducible and suitable for generating the formulation with the characters stated in *Rasashastra*. Pharmaceutical observations during processing and consistent product recovery were good, distinctive bright red colour suggesting effective completion of the pharmaceutical transformation. Standardisation of purification, *Kajjali* preparation, controlled heating and endpoint determination gives a consistent platform for quality assurance and future batch repeatability.

Significance of XRD Analysis

XRD examination showed that the main crystalline phase in the final formulation was mercury sulphide (HgS) in the form of cinnabar (α-HgS). The diffraction pattern was in good agreement with the standard ICDD reference pattern (PDF 00-006-0256). No extra crystalline impurity peaks were found.

These results demonstrate that the *Kupipakwa* procedure successfully converted the initial materials to crystalline α-HgS rather than leaving visible crystalline mercury or sulphur. This gives objective scientific proof for the physicochemical transformations stated in classical *Rasashastra*.

The lack of other crystalline phases also shows that the pharmaceutical process used resulted in a very uniform product with good phase purity, thereby supporting the reproducibility of the manufacturing process.

Significance of XRF Analysis

XRF elemental analysis showed that mercury and sulphur were the primary elemental constituents of the formulation with approximately 68.73 and 18.30%, respectively. These results are consistent with the expected composition of mercury sulphide produced during *Kupipakwa* processing.

Some other elements were found also, albeit in trace amounts and apparently from mineral source materials or natural impurities. The detection of arsenic and lead by XRF should be used with caution as XRF is a quick elemental screening technology and does not give the analytical sensitivity or quantitative accuracy of ICP-MS or ICP-OES for toxicological evaluation. Hence, additional ICP-based investigation is still required before any judgements can be drawn on the safety of the heavy metals.

The XRF results are in overall agreement with the effective synthesis of a sulphur-rich mercurial preparation commensurate with the expected medicinal content of conventional *Rasa Sindoor*.

Interpretation of Cytotoxic Activity

The present study found that the *ShadgunabalijaritaRasa Sindoor*had significant cytotoxic action on the human lung adenocarcinoma cell line, A-549.

The number of viable cells progressively decreased with increasing concentration of the formulation, demonstrating a definite biological response that was dosage dependent. Cell viability was lowered from 74.08% at 10 µg/mL to 40.06% at 100 µg/mL, with an estimated IC_{50} value of 76.41 µg/mL.

These findings were also complemented by morphological inspection in which treated cells displayed cellular shrinkage, rounding, diminished adherence and disruption of normal monolayer architecture, all hallmark characteristics of cytotoxic damage.

The formulation was less cytotoxic than the standard anticancer drug 5-fluorouracil (IC 50 = 7.88 µg/mL). However, the biological activity recorded still indicates that the formulation has appreciable antiproliferative potential that is worthy of further mechanistic investigation.

Correlation Between Classical Processing and Biological Activity

The present results are in general agreement with earlier pharmaceutico-analytical studies on *Rasa Sindoor*reporting crystalline α-HgS as the major phase after *Kupipakwa*processing. Similar studies have also confirmed mercury and sulphur as the major elemental ingredients therefore validating

the repeatability of classical pharmacological procedures. But very few publications are available for in-vitro cytotoxic action of *Shadgunabalijarita* Rasa Sindoor. thus the present inquiry is original.

Strengths of the Study

The present study involves ancient Ayurvedic drug manufacturing with modern analytical characterisation and biological evaluation. The *ShadgunabalijaritaRasa Sindoor*is fully characterised scientifically by standardised pharmaceutical processing, XRD phase identification, XRF elemental analysis and in-vitro cytotoxic assessment. Such comprehensive examination reinforces the scientific basis for future quality control and pharmaceutical investigations.

Limitations

The present study has certain limitations. Biological evaluation was restricted to an in-vitro model using a single human lung adenocarcinoma cell line, and the molecular mechanisms responsible for the observed cytotoxicity were not investigated. In addition, XRF provided elemental screening rather than definitive quantitative analysis of trace heavy metals. Therefore, ICP-based elemental analysis, mechanistic investigations, in-vivo studies and well-designed clinical studies are required before therapeutic conclusions can be drawn.

Future Perspectives

Future studies should investigate apoptosis, oxidative stress, mitochondrial dysfunction, cell-cycle arrest and molecular signalling pathways involved in the observed cytotoxic activity. Evaluation against additional cancer cell lines, normal cell lines and suitable animal models would further clarify the therapeutic potential and safety profile of *ShadgunabalijaritaRasa Sindoor*.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgements

The authors express their sincere gratitude to the *Department* of *Rasashastra* and*BhaishajyaKalpana*, Shri Ayurved College, Nagpur, for providing the necessary facilities, institutional support and academic guidance for conducting the present research. The authors also acknowledge the support extended during authentication of raw materials and express their gratitude to the analytical laboratory personnel for their valuable assistance in conducting physicochemical, microbiological and heavy metal analyses.

REFERENCE

1. Leiter A, Veluswamy RR, Wisnivesky JP. The global burden of lung cancer: current status and future trends. Nature Reviews Clinical Oncology. 2023;20(9):624-639. doi:10.1038/s41571-023-00798-3.
2. Sharma PV. Dravyaguna Vijnana. Vol. I. Varanasi: Chaukhambha Bharati Academy; 2013.
3. Sharma S. Rasatarangini. Edited by Kashinath Shastri. 11th ed. New Delhi: Motilal Banarsidass; 2014.
4. Sharma S. Rasatarangini. Edited by Kashinath Shastri. 11th ed. New Delhi: Motilal Banarsidass; 2014.
5. Shastri K. Rasatarangini. 11th ed. New Delhi: Motilal Banarsidass; 2014.
6. Mishra S. Ayurvediya Rasashastra. 11th ed. Varanasi: Chaukhambha Orientalia; 2011.
7. Subramanian P, et al. Anticancer property of gallic acid in A549, a human lung adenocarcinoma cell line, and possible mechanisms. Journal of Cancer Research and Therapeutics. 2010.
8. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1-2):55-63. doi:10.1016/0022-1759(83)90303-4.
9. Madhava. Ayurveda Prakasha. Edited by Gulraj Sharma Mishra. Varanasi: Chaukhambha Bharati. In.
10. Sharma S. Rasatarangini. Edited by Kashinath Shastri. 11th ed. New Delhi: Motilal Banarsidass; 2014.
11. Vagbhata. Rasa Ratna Samuccaya. Edited by Dattatreya Ananta Kulkarni. New Delhi: Meharchand Lachhmandas Publications; 2010.
12. Sharma S. Rasatarangini. Edited by Kashinath Shastri. 11th ed. New Delhi: Motilal Banarsidass; 2014.
13. Gupta NP, editor. Parada Samhita. Mumbai: Khemraj Shrikrishnadass Prakashan; Latest edition.
14. Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis. 7th ed. Boston: Cengage Learning; 2018.

15. Freshney RI. Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications. 7th ed. Hoboken (NJ): John Wiley & Sons; 2016.
16. The last Shadgunabalijarita Rasa Sindoor demonstrated organoleptic features that were indicative of adequate pharmaceutical processing. Formulation was brilliant red fine crystalline powder with smooth texture and free from apparent foreign materials. No evidence of any disagreeable smell or dampness was discovered indicating acceptable stability and suitable storage conditions.
17. Ministry of AYUSH, Government of India. The Ayurvedic Pharmacopoeia of India. Part I. New Delhi: Ministry of AYUSH; various volumes. In.