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PHARMACEUTICO-ANALYTICAL STUDY OF RASATALESHWAR RASA AND ITS IN VITRO CYTOTOXIC ACTIVITY ON A431 SKIN CANCER CELL LINE

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ABSTRACT

Skin cancer is one of the most common malignancies worldwide, and its incidence continues to rise due to increasing exposure to environmental and lifestyle-related risk factors. Despite advances in modern oncology, the search for safe and effective therapeutic agents remains an important area of research. Ayurveda describes several herbo-mineral formulations for the management of skin disorders, among which Rasataleshwar Rasa, a classical formulation mentioned in *Rasendrachintamani*, is indicated in skin conditions such as Vicharchika (eczema), Kandu (pruritus), and Kitibha (psoriasis). Considering the association between chronic inflammatory skin disorders and increased risk of skin malignancies, the present study was undertaken to evaluate the anticancer potential of Rasataleshwar Rasa. **Aim:** To evaluate the in vitro cytotoxic activity of Rasataleshwar Rasa against the A431 human skin carcinoma cell line. **Materials and Methods:** Rasataleshwar Rasa was prepared according to the classical method described in *Rasendrachintamani* and subjected to analytical evaluation. Its in vitro cytotoxic activity against the A431 skin carcinoma cell line was assessed using a standard cell viability assay, and the percentage of cell inhibition was determined at different concentrations. **Results:** Rasataleshwar Rasa exhibited concentration-dependent cytotoxic activity against the A431 skin carcinoma cell line, with a progressive

decrease in cell viability at increasing concentrations. The analytical evaluation further confirmed the quality and standardization of the prepared formulation. **Conclusion:** The findings suggest that Rasataleshwar Rasa possesses significant in vitro cytotoxic activity against A431 skin carcinoma cells and may serve as a potential complementary therapeutic agent for skin cancer. Further studies are required to establish its mechanism of action, safety, and clinical efficacy.

KEYWORDS: Ayurveda, Rasashastra, Rasataleshwar Rasa, Skin Cancer, A431 Cell Line, Cytotoxic Activity.

INTRODUCTION

Ayurveda, the traditional system of medicine of India, emphasizes a holistic approach to health and disease management through prevention, promotion, and treatment. Rasashastra is a specialized branch of Ayurveda that deals with the preparation and therapeutic application of herbo-mineral formulations known as Rasaushadhis.[1] These formulations are recognized for their rapid therapeutic action, lower dosage requirements, enhanced bioavailability, and broad clinical utility.[2] Among them, Kharaliya Rasayana is prepared through repeated trituration of medicinal ingredients, facilitating uniform mixing, reduction in particle size, and potentiation of therapeutic properties.

Rasataleshwar Rasa is a classical Kharaliya Rasayana described in *Rasendrachintamani* (Kushtharogadhikara). The formulation contains Shuddha Parad, Shuddha Gandhak, Shankha Bhasma, Shuddha Bhallataka, Shuddha Gunja, Haridra, Punarnava Moola, Apamarga Panchanga, Karanja Beeja, Ghritakumari, Arka Patra Swarasa, Maricha, Vidanga, and Gomutra. Owing to its Kapha-Vatahara properties, it is traditionally indicated in Vicharchika (eczema), Kandu (pruritus), and Kitibha Kushtha (psoriasis).[3]

Among these conditions, *Kitibha Kushtha* bears close resemblance to psoriasis, a chronic immune-mediated inflammatory skin disorder characterized by erythematous, scaly plaques resulting from abnormal keratinocyte proliferation. [4] Psoriasis affects approximately 0.2–4.8% of the global population and is associated with considerable physical and psychological morbidity.[5] Moreover, studies have reported an increased risk of non-melanoma skin cancers, particularly squamous cell carcinoma, among patients with severe psoriasis, partly attributable to long-term exposure to systemic therapies and phototherapy.[6] SCC is the second most common form of skin cancer and is characterized by hyperproliferation of epidermal

keratinocytes, presenting clinically as scaly erythematous plaques, ulcerative lesions, crusting, itching, and occasional bleeding.[7] Although current treatment modalities such as surgery, radiotherapy, and chemotherapy have improved patient outcomes, they are frequently associated with adverse effects including skin irritation, pigmentation changes, myelosuppression, increased susceptibility to infections, and peripheral neuropathy.

From an Ayurvedic perspective, the clinical manifestations of SCC indicate the involvement of Vata and Kapha Doshas. Therefore, formulations possessing Kapha-Vatahara, Kushthaghna properties may offer therapeutic benefits in such conditions. Several ingredients of Rasataleshwar Rasa, including Bhallataka, Haridra, Karanja, Punarnava, Maricha, Ghritakumari, Gomutra, and Arka, have been reported to possess anticancer, antioxidant, anti-inflammatory, and immunomodulatory activities. In addition, Kajjali, a major constituent of the formulation, has been reported to induce apoptosis and cellular morphological changes that may contribute to anticancer activity.[8]

Rasataleshwar Rasa is a relatively unexplored formulation, and no scientific studies evaluating its anticancer potential have been reported to date. Since it is a newly investigated formulation, preliminary in vitro evaluation is essential to assess its therapeutic efficacy before further experimental or clinical studies. Cell line studies provide a reliable, reproducible, and cost-effective platform for screening potential anticancer agents. Therefore, the present study was undertaken to prepare and standardize Rasataleshwar Rasa according to classical references and to evaluate its in vitro cytotoxic activity against the A431 human skin carcinoma cell line. The findings of this study may provide a scientific basis for the therapeutic potential of Rasataleshwar Rasa and serve as a foundation for future pharmacological and clinical investigations.

AIM

To prepare *Rasataleshwar Rasa*, analyze it, and study its in vitro cytotoxic action on skin carcinoma using the A431 cell line.

OBJECTIVES

Primary Objective

1. To prepare *Rasataleshwar Rasa* w.s.r. to *Rasendrachintamani*.

Secondary Objectives

1. To identify and authenticate raw materials of *Rasataleshwar Rasa*.
2. To analyse *Rasataleshwar Rasa* physico-chemically.
3. To evaluate in vitro cytotoxic action of *Rasataleshwar Rasa* on skin carcinoma using A431 cell line.
4. To study literature review of *Rasataleshwar Rasa* and its contents in detail.
5. To study literature review of *Kitibha Kushtha*(Psoriasis) and Squamous cell carcinoma.
6. To study literature review of Cell line study and A431 cell line.

MATERIALS AND METHODS

Materials

The raw materials, including Parad, Gandhak, Shankha, and other associated ingredients, were procured from local market, after confirming their Grahya Lakshanas (acceptable quality parameters).

Physico-chemical and instrumental analysis were conducted at Laboratory.

Methods Adopted

The Pharmaceutical study was carried out under the following steps

1. Parada Shodhana[9]

Reference- Parad Samhita 30/85.

Procedure

- 150 g Ashuddha Parada was taken in a clean Khalva Yantra.
- Bhavana was given with freshly prepared Rason Swarasa for 7 days (42 hrs total), using 150 g Swarasa.
- After trituration, Kshalana was performed with hot water to remove impurities.
- Purified Parada was finally collected by filtering through a clean cloth.

2. Gandhaka Shodhana (Purification of Sulphur)[10]

Reference- Rasa Ratna Samucchya 3/20.

Procedure

- Gandhaka was coarsely powdered in a clean Khalva Yantra.
- It was melted in ghee and filtered through a cloth into milk.

- The Gandhaka solidified upon contact with milk, after which it was thoroughly washed with warm water, patted dry, and re-pounded into powder.
- This entire procedure was repeated thrice for complete purification.

3. Shankha Shodhana[11]

Reference- Rasa Tarangini 12/6-7.

Procedure

- Ashuddha Shankha was weighed, wrapped in muslin cloth, and tied as a Pottali.
- The Pottali was suspended in a Dolayantra setup containing Nimbu Swarasa.
- It was heated mildly for 12 hours, undergo Swedana (sudation) with fresh Nimbu Swarasa added intermittently.
- After cooling, Shankha pieces were washed with warm water and dried under sunlight.

4. Shankha Bhasma Preparation[12]

Reference - Rasa Tarangini 12/17-19.

Procedure

- Shodhita Shankha was placed in a Sharava Samputa, sealed with Kapadmitti, and sun-dried.
- The Samputa was subjected to Gajaputa (700–900 °C for 1 hr), then allowed to cool naturally. Calcined material was collected, triturated with Ghritkumari Swarasa, and prepared into Chakrikas.
- Chakrikas were dried, sealed again in Samputa, and given a second Gajaputa under the same conditions.
- After natural cooling, the product was collected and weighed; the process was repeated for a third Gajaputa.
- Final Shankha Bhasma was obtained after three successive Gajaputas

5. Bhallataka shodhan[13]

Reference- Rasa Tarangini 24/477- 478.

Procedure

- Ripened seeds were collected; only those that sank in water were selected.
- Caps were removed, seeds cut into halves, and mixed with Ishtika Churna (brick

powder).

- The mixture was tied in a Pottali and rubbed for 7 days, replacing brick powder 2–3 times.
- Toxic oil was absorbed by the brick powder; seeds were washed thoroughly with hot water.
- Finally, seeds were dried, yielding purified (Shuddha) Bhallataka safe for use.

6. Gunja Shodhana[14]

Reference- Rasa Tarangini 24/443-444.

Procedure

- Raw Gunja seeds were weighed, tied in a muslin Pottali, and immersed in fresh cow's milk (Dolayantra method)
- The vessel was heated mildly for 6 hours, with milk replenished as needed.
- After cooling, the Pottali was removed, and seeds were washed with warm water.
- The seed coat was peeled off and seeds shade-dried and then grounded into fine powder.

7. Preparation of Churna of other ingredients[15]

Reference- Sharangdhar Samhita Madhyam Khand 6/1.

Procedure

- Gunja, Bhallataka, and other ingredients (Haridra, Apamargapanchang, Punarnava mool, Vidanga, Maricha, Karanja beej) were separately powdered after Shodhana, accurately weighed, and ground in small batches.

8. Preparation of Kajjali[16]

Reference- Rasa Ratna Samucchaya 8/5.

Procedure

- Equal parts of Shuddha Parada and Shuddha Gandhaka were triturated in a Khalva Yantra until a homogeneous Kajjali was obtained, showing classical characteristics of a fine, black, smooth, and lusterless powder.

9. Preparation of Rasataleshwar rasa[17]

गुंजाशंखकरंजचूर्णरजनीभल्लातकबर्हिःशिखा
कन्यासूर्यपयः पुनर्नवरजो गंधस्तथा सूतकम् ।
गोमूत्रेण श्रुतं विड़ंगमरीचैः क्षौद्रंच
तत्तुल्यकं हन्यादाशुविचर्चिकारूजमिदंकण्डूं तथा कैटिभम् ।। - र. चि. कुष्ठ ९/७८

Reference:- Rasendrachintamani Kushtharogadhikar

Apparatus used:- Weighing machine, Spoons, Spatula/Tongs, Measuring cylinders, Khalva yantra (mortar with pestle) Stainless steel vessels, Grinder & Sieves (of different mesh size), Glass container, Stove and Gas cylinder, cloth, plate.

Ingredients

- Shuddha Parada – 15 gms
- Shuddha Gandhak - 15 gms
- Shuddha Gunja beej - 15 gms
- Shuddha Bhallatak - 15 gms
- Shankh Bhasma (Conch shell) - 15 gms
- Karanja beej - 15 gms
- Haridra - 15 gms
- Apamarga panchanga - 15 gms
- Punarnava mool - 15 gms
- Maricha - 15 gms
- Vidanga – 15 gms
- Ghritkumari – 330 ml
- Ark ksheer – 130 ml
- Gomutra - 8 times of overall drug i.e 2168 ml

Procedure

- Kajjali was mixed thoroughly with Gunja Beej, Karanja Beej, Haridra, Bhallataka, Apamarga panchang, Punarnava Mool, Shankh Bhasma, Vidanga, and Maricha powders.
- Bhavana was performed using Ghritkumari Swarasa and Ark Ksheer.
- The mixture was added to Gomutra (8 times its quantity) in a stainless steel vessel.
- The vessel was heated over Mandagni (mild flame) until Gomutra was completely absorbed.
- The preparation was spread on a tray, dried under sunlight, finely powdered, and stored in a dry container.

10. Physico-Chemical Analysis

- **pH:** Measures the acidity or alkalinity of the formulation.
- **Moisture Content (Loss on drying at 110°C):** Determines the amount of water or

volatile matter present in the sample.

- **Solubility in Water:** Assesses how much of the formulation dissolves in water.
- **Total Ash Value:** Represents the total inorganic residue remaining after complete combustion of the sample.
- **Acid Insoluble Ash:** Measures the amount of silica and acid-insoluble matter in the total ash.
- **Alcohol Soluble Extractive:** Indicates the quantity of active constituents soluble in alcohol.
- **Water Soluble Extractive:** Indicates the quantity of active constituents soluble in water.
- **Particle Size:** Refers to the average size of particles in the powdered formulation, often affecting bioavailability.
- **XRD (X-ray Diffraction):** Identifies the crystalline structure and phase composition of the sample.
- **XRF (X-ray Fluorescence):** Determines the elemental composition and concentration in the sample.
- **EDX (Energy Dispersive X-ray Analysis):** Provides qualitative and semi-quantitative elemental analysis of the sample surface.

11. Experimental Study –

Materials

1. Cell Line

- A431 human epidermoid carcinoma cell line.

2. Cell Culture Reagents

- Dulbecco's Modified Eagle Medium (DMEM High Glucose; #AL007A, HiMedia)
- Foetal Bovine Serum (FBS; #RM10432, HiMedia)
- MTT Reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (#4060, HiMedia)

3. Laboratory instruments

- 96-well culture plates
- T25 cell culture flasks (#12556009, Biolite-Thermo)
- 50 mL centrifuge tubes (#546043, Tarsons)
- 1.5 mL centrifuge tubes (Tarsons)
- 10 mL serological pipettes (Tarsons)

- Micropipette tips (10–1000 µL, Tarsons)

4. Equipment

- Refrigerated centrifuge (Eppendorf 5430R)
- Micropipettes (2–10 µL, 10–100 µL, and 100–1000 µL)
- Inverted microscope (Metzer)
- CO_2 incubator maintained at 37°C with 5% CO_2 and humidified atmosphere (Thermo Fisher Scientific)
- Microplate reader (Biotek Synergy H1)

5. Methodology[18,19,20]

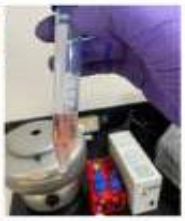
Cell Viability Assay (MTT Assay)

The in vitro cytotoxic activity of Rasataleshwar Rasa against the A431 human epidermoid carcinoma cell line was evaluated using the MTT assay. A431 cells were seeded into a 96-well plate at a density of 5×10^3 cells/well in DMEM supplemented with 10% FBS and allowed to adhere. The cells were then treated with different concentrations of Rasataleshwar Rasa (62.5, 125, 250, 500, and 1000 µg/mL) and incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 48 hours.

Following incubation, the culture medium was removed and MTT solution was added to each well to obtain a final concentration of 0.5 mg/mL. The plate was protected from light and incubated for an additional 3 hours to allow the formation of formazan crystals. Subsequently, the MTT solution was discarded and 100 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the crystals. The contents were mixed thoroughly, and the absorbance was measured at 570 nm using a microplate reader with 630 nm as the reference wavelength. Cell viability and percentage cytotoxicity were calculated based on the absorbance values obtained.

Cell Line Experiment






Reviving cryopreserved A431 cells

Adding media to cells in centrifuge tube

Cell pellet formed after Centrifugation

Cell pellet is resuspended in fresh media for culturing and kept for incubation for 24 hours at 37°C in a 5% CO_2 incubator.


Cell suspension in 96 well plate with DMEM + FBS kept for incubation to grow cells

Testing compound – Rasataleshwar Rasa

Cells treated with the test compound at different concentrations are incubated for 48 hours at 37°C in a 5% CO_2 incubator.

After incubation, MTT reagent added to each well, followed by incubation to form formazan crystal, Solubilizing the formazan crystals with DMSO.

Measuring absorbance to assess cell viability using Microplate reader, based on the amount of solubilized formazan.

OBSERVATION AND RESULT

1. Parada Shodhana

- Initial Weight: 150 g
- Final Weight: 135.76 g
- Parada became shiny, clear, and impurity-free after washing.

2. Gandhaka Shodhana

- Initial Weight: 150 g
- Final Weight: 144 g
- Colour of Gandhaka changed from yellow to light greenish-yellow; solidified immediately on contact with cow's milk.

3. Shankha Shodhana

- Initial Weight: 245 g
- Final Weight: 215 g
- Surface roughness increased; Nimbu Swarasa colour changed from greenish-yellow to brown.

4. Shankha Bhasma

- Initial Shuddha Shankha Weight: 215 g
- Weight of *Shankha bhasma* after 1st *Gajaputa*: 198.61 g.
- Weight of *Shankha Chakrika* after 1st *Bhavana*: 227 g.
- Weight of *Shankha bhasma* after 2nd *Gajaputa*: 182 g.
- Weight of *Shankha Chakrika* after 2nd *Bhavana*: 202 g.
- Final Weight (After 3rd Gajaputa): 180.37 g
- Achieved fine powder with white colour, characteristic Aloe vera smell, and slightly alkaline pH (~10).

5. Bhallataka Shodhana

- Initial Weight: 99.23 g
- Final Weight: 93 g
- Seeds became less sticky, non-greasy, dull black after processing.

6. Gunja Shodhana

- Initial Weight: 88.85 g

- Final Weight: 73 g
- Red seed coat turned dull brown; outer coat loosened and removed, Godugdha colour changed from white to brown, indicating extraction of toxic elements.

7. Preparation of Churna (Herbal Powders)

Sr.No.	Ingredient	Initial Weight (g)	Fine Powder Obtained (g)
1.	Karanja seeds	80	70
2.	Haridra	80	70
3.	Apamarga panchanga	80	65
4.	Punarnava mool	80	60
5.	Maricha	80	75
6.	Vidanga	80	70

- smooth, fine-textured powder with characteristic colour and odour achieved after sieving.

8. Preparation of Kajjali

- Total Kajjali Weights: Shuddha Parada and Gandhaka (Equal parts i.e 15g each) i.e 30g.
- Colour changed from yellowish-green → greyish → black.
- Final Kajjali showed proper Rekhapurnatva, Nishchandratva, and Varitaratva tests.

9. Observations During Preparation of Rasataleshwar rasa

- The powdered ingredients were added sequentially into the mortar and triturated for approximately 20 minutes to obtain a homogeneous mixture.
- The first Bhavana was carried out using 330 mL of Ghritakumari Swarasa for 210 minutes, resulting in a smooth, dark greenish-black formulation with a slightly metallic odour.
- The second Bhavana was performed using 130 mL of Arka Patra Swarasa for 270 minutes, producing a smooth, dark black formulation with a characteristic odour.
- Initial coarseness due to ingredients such as Bhallataka, Apamarga Panchanga, and Punarnava Moola Churna gradually reduced with continuous Mardana, yielding a smooth texture.
- The Bhavita mixture was processed with 2168 mL of Gomutra for approximately 4½ hours until complete absorption, after which it developed a characteristic Gomutra odour and greenish-black colour.
- After sun drying, pulverization, and sieving, the final product obtained was a fine, smooth, greyish-black powder with a characteristic odour.
- The initial weight of the powdered ingredients was 165 g, which increased to 271 g after

Bhavana due to absorption of the liquid media.

- The final yield of Rasataleshwar Rasa obtained was 238 g, with minor weight loss attributed to evaporation, adhesion to equipment, and handling during processing.

10. Analytical result

Table No. 1: Showing results of Organoleptic characteristic of Rasataleshwar rasa

Organoleptic Characteristics	Rasataleshwar Rasa
Form/Consistency	Fine Powder form
Colour	Greyish Black
Odour	Characteristic (Prominent of Gomutra)
Taste	Characteristic
Touch	Fine and Smooth

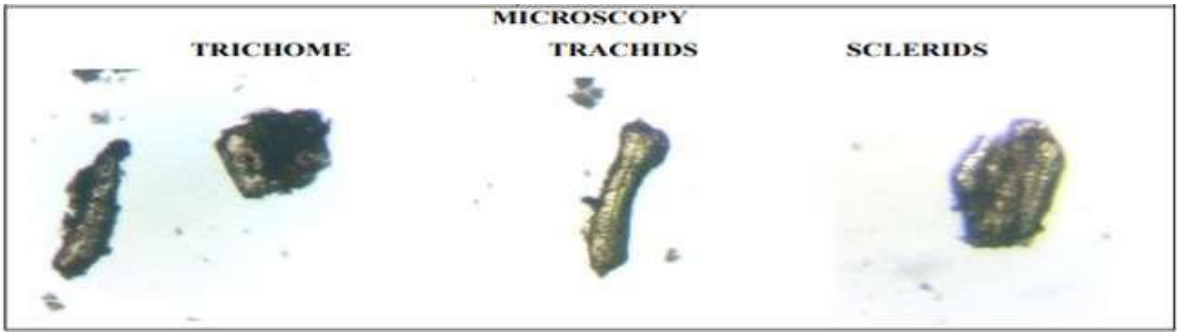
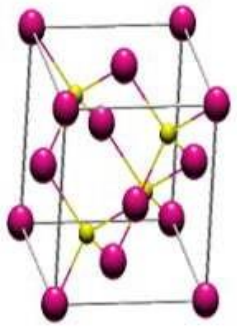


Table No. 2: Showing results of Physico-chemical analysis of Rasataleshwar rasa

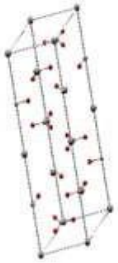
Physico-chemical Parameters	Rasataleshwar Rasa
pH	5.4
Moisture content (Loss on drying at 110 degree Celsius)	4.0%
Solubility in Water	Sparingly soluble in water
Total Ash Value	10.22%
Acid Insoluble Ash	3.0%
Alcohol Soluble Extractive	16.37%
Water Soluble Extractive	6.02%
Particle Size	Particle size - 5216.1 nm, Polydispersity Index (PDI) of 1.079 nm Z-average particle size of 1429.6 nm.
XRD (X-ray Diffraction)	Peaks of Metacinnabar (HgS) and Calcite ($CaCO_3$).
XRF (X-ray fluorescence)	48.6% of Sulphur trioxide due to presence of Gandhaka. 15.8% of Mercuric Oxide due to presence of Parada. 15.0% of Calcium oxide due to presence of Shankha Bhasma. 9.41% of Potassium Oxide, 4.67% of Silicon Dioxide, 3.37% of Phosphorus Pentoxide, 2.02% of Chlorine and other trace minerals were also seen.
EDX	Showing most prominent Energy peaks of Mercury (Hg), Sulphur (S), Phosphorus (P), Chlorine (Cl), and Silicon (Si),

Rasataleshwar rasa

Crystal structure view: Metacinnabar (Hg S)



Crystal structure view: Calcium Carbonate (CaCO3)



11. Experimental Study: Observations and Results

- The MTT assay was performed in triplicate on 19/06/2025 to evaluate the in vitro cytotoxic activity of Rasataleshwar Rasa against the A431 human epidermoid carcinoma cell line.
- Statistical analysis was carried out using GraphPad Prism software, and the results were expressed as mean values.
- Rasataleshwar Rasa exhibited a dose-dependent cytotoxic effect, with cell viability decreasing as the concentration of the formulation increased.
- The mean percentage of cell viability observed at different concentrations was as follows:

o 62.5 µg/mL: 88.6%
o 125 µg/mL: 83.9%
o 250 µg/mL: 72.55%
o 500 µg/mL: 28.23%
o 1000 µg/mL: 18.6%

- A progressive decline in absorbance values was observed with increasing concentrations, indicating reduced cellular metabolic activity.
- The absorbance value was 1.548 at 62.5 µg/mL and decreased to 0.34 ± 0.02 at 1000 µg/mL.
- The dose-response curve demonstrated a characteristic sigmoidal pattern, confirming standard cytotoxic activity.
- The calculated IC_{50} value of Rasataleshwar Rasa was 278.6 µg/mL, indicating moderate

cytotoxic potential against A431 cells.

- The coefficient of determination (R^2) was 0.6829, indicating an acceptable fit to the nonlinear regression model.
- The findings suggest that Rasataleshwar Rasa exhibits significant concentration-dependent cytotoxic activity, with pronounced effects observed at concentrations above 250 µg/mL.
- These results support the potential anticancer efficacy of Rasataleshwar Rasa and warrant further evaluation through advanced in vitro and in vivo studies.

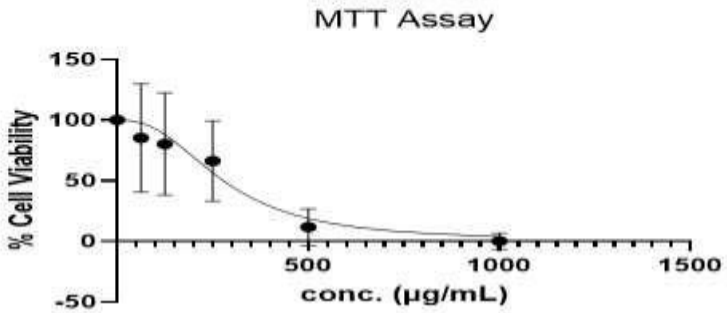
Table 4: Percentage cell viability by the treatments

Treatments	Conc. (µg/mL)	% cell viability (n1)	% cell viability (n2)	% cell viability (n3)
Test Formulation	1000	23.2944069	20.0527704	12.5707814
Test Formulation	500	39.7049785	29.8812665	15.1377878
Test Formulation	250	95.697603	79.1556728	42.808607
Test Formulation	125	83.0362631	118.601583	50.0943752
Test Formulation	62.5	106.392133	111.543536	46.1683654
DMEM+ Cell	-	100	100	100

Table 3: Corrected absorbance after MTT assay

Treatments	Conc. (µg/mL)	Abs (n1)	Abs (n2)	Abs (n3)
Test Formulation	1000	0.379	0.304	0.333
Test Formulation	500	0.646	0.453	0.401
Test Formulation	250	1.557	1.2	1.134
Test Formulation	125	1.351	1.798	1.327
Test Formulation	62.5	1.731	1.691	1.223
DMEM+ Cell	-	1.627	1.516	2.649
DMEM	-	0	0	0

[Inhibitor] vs. normalized response -- Variable slope	
Best-fit values	
IC50	**278.6**
HillSlope	-2.491
logIC50	2.445
95% CI (profile likelihood)	
IC50	165.7 to 420.8
HillSlope	??? to -0.9564
logIC50	2.219 to 2.624
Goodness of Fit	
Degrees of Freedom	16
R squared	0.6829
Sum of Squares	11417
Sy.x	26.71
Constraints	
IC50	IC50 > 0
Number of points	
# of X values	18
# Y values analyzed	18



DISCUSSION

The present study evaluated Rasataleshwar Rasa through pharmaceutical preparation, analytical standardization, and in vitro cytotoxic assessment against the A431 human epidermoid carcinoma cell line. The formulation was prepared according to classical Ayurvedic references using authenticated raw materials subjected to appropriate Shodhana procedures to enhance safety and therapeutic efficacy. During pharmaceutical processing, Bhavana with Ghritakumari Swarasa and Arka Patra Swarasa, followed by Gomutra Paka, facilitated the incorporation of phytoconstituents and inorganic components into the formulation, which may contribute to its therapeutic potential. Analytical evaluation confirmed the quality and standardization of Rasataleshwar Rasa, characterized as a fine greyish-black powder with Gomutragandhi odour, acidic pH, low moisture content, and acceptable ash and extractive values. XRD analysis demonstrated the presence of HgS (Metacinnabar) and $CaCO_3$ (Calcite), while XRF and EDX analyses confirmed the presence of major elements such as mercury, sulphur, calcium, phosphorus, chlorine, and silicon, validating the composition of the formulation. Particle size analysis revealed nanoscale dimensions, which may enhance bioavailability and cellular interaction. The MTT assay demonstrated a concentration-dependent cytotoxic effect of Rasataleshwar Rasa against A431 cells, with an IC_{50} value

of

278.6 µg/mL, indicating moderate anticancer potential. The observed cytotoxic activity may be attributed to the synergistic action of ingredients such as Bhallataka, Haridra, Karanja, and Kajjali, which have been reported to possess antiproliferative, antioxidant, apoptotic, and anticancer properties. The characteristic sigmoidal dose-response curve further supported the reliability of the cytotoxic findings. Thus, the results suggest that Rasataleshwar Rasa possesses promising in vitro anticancer activity against skin carcinoma cells. However, further studies, advanced in vitro assays, and in vivo investigations are warranted to establish its safety profile, mechanism of action, and therapeutic applicability in the management of skin cancer.

CONCLUSION

Rasataleshwar Rasa was successfully prepared according to the classical reference of Rasendrachintamani (Kushtharogadhikara 9/78) following standard pharmaceutical procedures. Since no previous scientific studies on this formulation were identified, the present work provides preliminary pharmaceutical, analytical, and biological data for this classical preparation. All raw materials were authenticated, and the purification (Shodhana) procedures of Parada, Gandhaka, Shankha, Bhallataka, and Gunja were carried out as per classical references. Shankha Bhasma was prepared through three consecutive Gajaputa cycles, and Kajjali was prepared successfully, followed by sequential Bhavana with Ghritakumari Swarasa and Arka Patra Swarasa, and Gomutra Paka, yielding a fine greyish-black powder with characteristic odour.

Analytical evaluation established baseline physicochemical characteristics of Rasataleshwar Rasa. Advanced analytical studies including XRD, XRF, and EDX confirmed the presence of HgS (Metacinnabar), $CaCO_3$ (Calcite), and various mineral constituents, indicating the chemical transformations occurring during pharmaceutical processing.

The in vitro cytotoxicity assessment demonstrated dose-dependent activity of Rasataleshwar Rasa against the A431 human epidermoid carcinoma cell line, with significant cytotoxic effects observed at concentrations of 250, 500, and 1000 µg/mL. These findings suggest that Rasataleshwar Rasa possesses promising anticancer potential and warrant further detailed in vitro and in vivo investigations to elucidate its safety, efficacy, and mechanism of action.

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