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

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ABSTRACT

Introduction: Skin cancer is among the most common malignancies worldwide, and squamous cell carcinoma constitutes a major type of non-melanoma skin cancer. In Ayurveda, most dermatological disorders are described under the broad concept of Kushtha. Rasamanikya, a classical Ayurvedic herbo-mineral formulation prepared from Shodhita Hartala, is indicated in disorders such as Kushtha, Vicharchika, Nadi Vrana, and Dustha Vrana. Therefore, the present study was undertaken to evaluate the in vitro cytotoxic activity of Rasamanikya on A431 skin cancer cell line. **Aim:** To prepare and analyze Rasamanikya according to Rasendra Chintamani and to study its in vitro cytotoxic activity on A431 skin cancer cell line. **Materials and Methods:** Rasamanikya was prepared from Shodhita Hartala processed in Kushmanda Swarasa and Amla Dadhi as per the classical method mentioned in Rasendra Chintamani using Antardhoom procedure in closed Sharava. The prepared formulation was subjected to organoleptic, physicochemical, X-ray fluorescence (XRF), X-ray diffraction (XRD), and particle size analysis. In vitro cytotoxic activity was assessed on A431 human skin cancer cell line using MTT assay at concentrations of 1000, 500, 250, 125, and 62.5µg/mL. **Results:** Rasamanikya was obtained as a light yellowish-colored, odorless,

tasteless fine powder. Physicochemical analysis showed moisture content 0.16%, total ash 3.16%, acid insoluble ash 1.96%, water soluble extractive 1.46%, alcohol soluble extractive 1.55%, pH 5.6, specific gravity 0.998, and volatile matter 96.67%. XRF analysis revealed arsenic (64.4%) and sulphur (28.3%) as major constituents. XRD analysis demonstrated transformation of raw Hartala from orpiment (As_2S_3) to dimorphite (As_4S_3) form in Rasamanikya. Particle size analysis showed a primary particle size around 385 nm with Z-average of 686 nm and polydispersity index of 0.602. The MTT assay demonstrated dose-dependent cytotoxic activity against A431 cell line with progressive reduction in cell viability at increasing concentrations. Rasamanikya exhibited an IC_{50} value of 197.6 µg/mL. **Conclusion:** The present study establishes successful preparation and analytical standardization of Rasamanikya. The formulation exhibited significant dose-dependent cytotoxic activity against A431 skin cancer cell line, indicating its potential anticancer activity. Further in vivo studies and mechanistic investigations are required to validate its therapeutic applicability in skin cancer management.

KEYWORDS: Rasamanikya, Hartala, A431 cell line, Skin cancer, Cytotoxic activity, MTT assay, Rasashastra.

INTRODUCTION

Ayurveda is a traditional system of medicine that focuses on maintaining health and treating disease through a holistic approach. Medicinal therapy (Aushadha) is one of the fundamental components of Ayurvedic treatment. The preparation of medicines is governed by two major branches: Rasashastra, which deals with mineral and metallic drugs, and Bhaishajya Kalpana, which focuses on herbal formulations. In Rasashastra, specific pharmaceutical procedures such as Shodhana and Marana are employed to convert raw minerals into safe, bioavailable, and therapeutically effective forms.

Ayurvedic pharmaceutics includes a wide range of compound formulations prepared from plant, mineral, and animal sources and presented in various dosage forms. Mineral-based preparations are especially valued for their potency and rapid therapeutic action. Rasamanikya, a classical formulation prepared from purified Haritala (orpiment), is traditionally used in the management of several skin disorders. Various methods for its preparation are described in classical texts; for the present study, the procedure mentioned in Rasendra Chintamani was followed.

Skin diseases represent a major health concern worldwide, often causing chronic discomfort

and psychological distress. In Ayurveda, a broad spectrum of skin disorders is described under Kushtha Roga, which is further classified into Maha Kushtha and Kshudra Kushtha. Although the classical descriptions do not directly correspond to modern dermatological terminology, many clinical features of chronic skin diseases can be correlated. The skin (Twacha) is regarded as the body's first line of defense, and its pathological changes are well documented in Ayurvedic literature.

Skin cancer is among the most common cancers worldwide, with increasing incidence due to ultraviolet radiation exposure, unhealthy lifestyle, processed foods, and other environmental factors. In Ayurveda, various formulations are described for the management of skin disorders. Rasamanikya, a classical formulation prepared from Shodhita Hartala, is traditionally used in different dermatological conditions. The present study was undertaken to evaluate the pharmaceutico-analytical profile and in vitro cytotoxic activity of Rasamanikya on A431 skin cancer cell line.

Rationale of the study

Rasamanikya is a classical Ayurvedic formulation regularly used in various skin disorders; however, its cytotoxic potential against skin cancer has not been scientifically validated. Being a comparatively cost-effective mineral formulation, it may offer a potential alternative approach in cancer research. Hence, the present study was undertaken to establish its pharmaceutico-analytical profile and evaluate its in vitro cytotoxic activity on A431 skin cancer cell line.

AIMS AND OBJECTIVES

Aim

To prepare and analyze Rasamanikya and to study its cytotoxic activity on A431 skin cancer cell line (in-vitro).

Objectives

Primary Objectives

1. To prepare Rasamanikya as mentioned in Rasendrachintamani.

Secondary Objectives

1. To identify and authenticate raw materials.
2. To analyze Rasamanikya physico-chemically.

3. To evaluate the cytotoxic action of Rasamanikya on the skin cancer cell line.

MATERIAL AND METHODS

The entire study is divided into three stages

A. Pharmaceutical work i.e. Preparation of rasamanikya
B. Analytical study
C. Experimental study i.e. In vitro cell line study

A. Pharmaceutical work i.e. Preparation of rasamanikya

1) Collection of raw materials

Hartal and kushmand were bought from reliable place.

Fresh godugdha was procured from nearby cattle shed for preparation of dadhi.

2) Authentication of Raw Materials

Hartal, kushmand, dadhi were authenticated from a reliable and recognized laboratory.

3) Shodhan of Hartala

तालकं वंशपत्राख्यं कूष्माण्डसलिले क्षिपेत् ।

सप्तधा वा त्रिधा वाऽपि दध्नाऽम्लेन तथैव च।।

शोधयित्वा पुनः शुष्कं चूर्णयेत्तण्डुलाकृति ।

Rasendrachintamani 9/128-133 [1]

In the preparation of rasamanikya, the very first important step is to purify hartala. The purification is carried out in two stages, using the kshipta (immersion) method:

First stage – shodhan in kushmanda swarasa

Second stage – shodhan in dadhi

3a. preparation of kushmand swaras

Ingredients: kushmand fruit

Equipment: knife, plate, mixer grinder, cloth, weighing machine, container, measuring cylinder.

Procedure: For the extraction of kushmand swaras, the kushmand is first cut into small pieces and collected in a clean plate. Nearly 1 kg of fresh fruit is taken for this purpose. The swaras is obtained by squeezing and then filtered through a clean cloth to separate the liquid from the

fibrous residue. The clear swaras is then transferred into a glass container and used for subsequent experimental use.

Observations: The freshly prepared kushmand swaras appeared cream in color with a watery consistency.

Precautions

1) All equipment must be clean and free from any contamination.

2) Everyday new kushmand swaras is used

3) The obtained swaras was carefully filtered through a washed white cloth to ensure purity and to prevent contamination.

Result: from the processing of fresh kushmand fruits, about 1 litre of juice was obtained.

3b. Shodhan of hartala in kushmanda swaras

Ingredients: kushmand swaras, hartala

Equipment: plate, spoon, cloth, weighing machine, container, measuring cylinder, khalva yantra (mortar and pestle)

Name of shodhan process: kshipta method(immersion)

Procedure

1. Raw hartala was first broken into small pieces with the help of a mortar and pestle.
2. The pieces were placed in a clean vessel.
3. Freshly prepared kushmand swaras was poured into the container until the drug was completely immersed.
4. The immersion was maintained for 24 hours.
5. After 24 hours, the used swaras was discarded and replaced with freshly extracted swaras.
6. Steps 3–5 were repeated daily with fresh juice for seven consecutive days.
7. At the end of the seventh day, the purified hartala was taken out, washed properly, and dried for further use.

Precautions

1. A glass beaker was used for the kshipta process to prevent any possible reaction of hartala with metals.
2. Care was taken while changing the kushmand swaras to avoid accidental loss of hartala particles.

3. Before adding fresh swaras each day, the hartala pieces were gently washed with lukewarm water.
4. The wash water was carefully decanted from the glass beaker to ensure that no portion of the drug was lost.

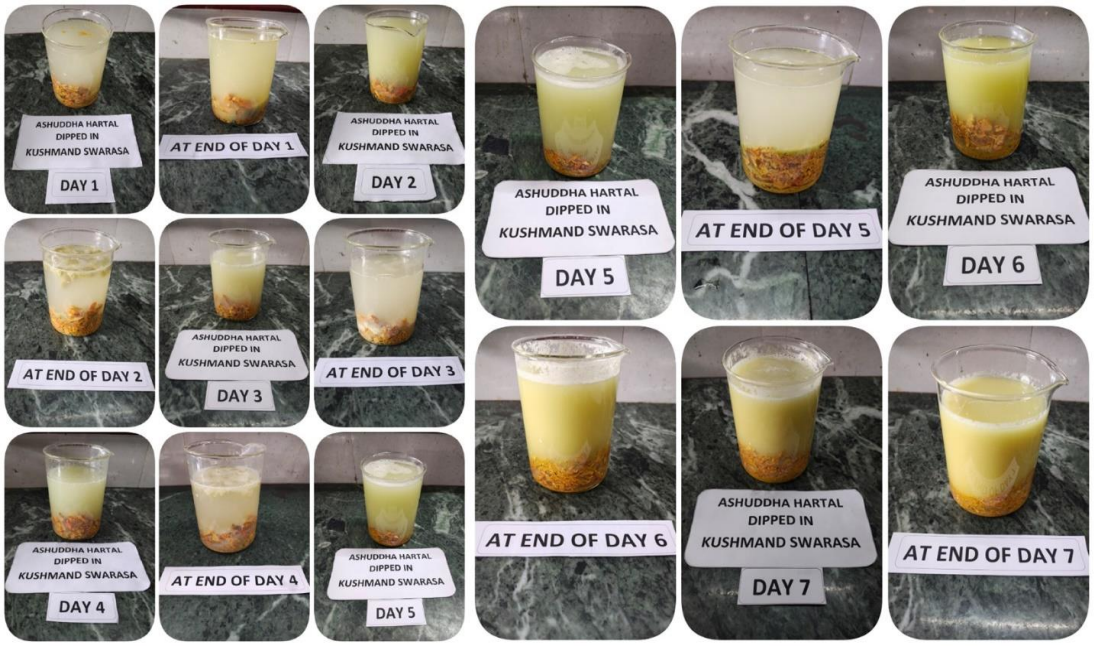


Figure 1: Shodhan of Hartala in Kushmanda swaras.

3c. Preparation of dadhi

Ingredients: godugdha (cow milk)

Equipment: Container, spoon, measuring cylinder, weighing machine, gas stove

Procedure

1. Milk Selection - fresh, good quality cow milk is selected.
2. Heating – Milk is boiled and stirred occasionally to prevent it from sticking to the bottom.
3. Cooling – Then boiled milk is allowed to cool to a lukewarm temperature
4. Inoculation – 1 to 2 teaspoons of curd added to the warm milk.
5. Fermenting the Milk – Milk is Covered by the container with a lid and allowed to ferment.

Precautions

1. Take Clean utensils to ensure no contamination that might hinder fermentation.
2. Consistency of temperature during fermentation is crucial for a good result.

3d. Shodhan of hartala in dadhi

Ingredients: dadhi, shodhit hartala in kushmand swaras

Equipment: plate, spoon, cloth, weighing machine, container, measuring cylinder, khalva yantra (mortar and pestle), gas stove.

Name of Shodhan Process: Kshipta method(immersion)

Procedure

1. Shodhit Hartala in kushmand swaras were placed in a clean vessel.
2. Freshly prepared dadhi was poured into the Container until the drug was completely immersed.
3. The immersion was maintained for 24 hours.
4. After 24 hours, the used dadhi was discarded and replaced with fresh dadhi.
5. Steps 2 to 4 were repeated daily with fresh dadhi for seven consecutive days.
6. At the end of the seventh day, the purified hartala was taken out, washed properly, and dried for further use.

Precautions

1. A Container was used for the Kshipta process to prevent any possible reaction of hartala.
2. Care was taken while changing the dadhi to avoid accidental loss of hartala particles.
3. Before adding fresh dadhi each day, the hartala pieces were gently washed with lukewarm water.
4. During washing, the used water was carefully removed from the container with the help of a clean muslin cloth to ensure that no drug particles were lost.

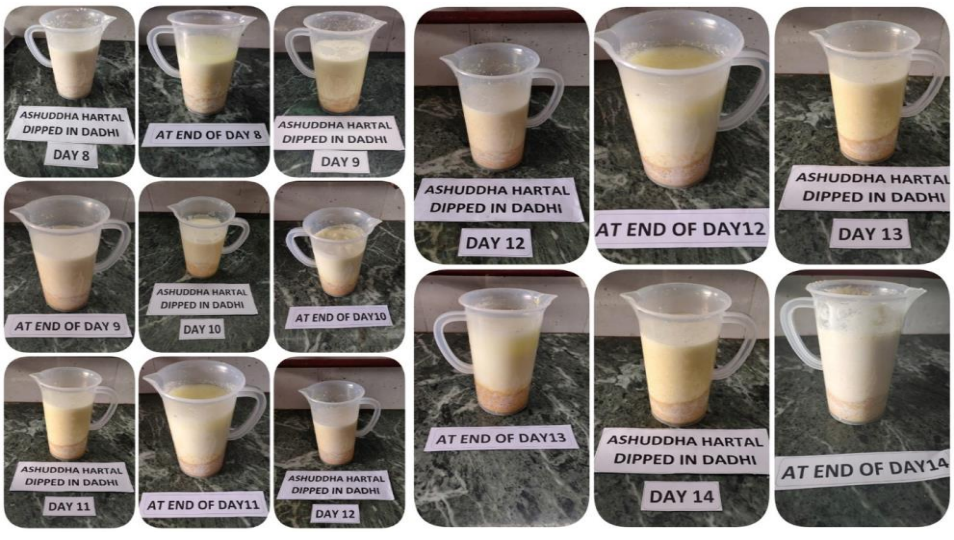


Figure 2: Shodhan of hartala in dadhi.

4) Preparation of Rasamanikya

तालकं वंशपत्राख्यं कूष्माण्डसलिले क्षिपेत् ।

सप्तधा वा त्रिधा वाऽपि दध्नाऽम्लेन तथैव च।।

शोधयित्वा पुनः शुष्कं चूर्णयेत्तण्डुलाकृति ।

ततः शरावके पात्रे स्थापयेत्कुशलो भिषक् ।।

बदरीपत्रकल्केन सन्धिलेपञ्च कारयेत् ।

अरुणाभं हयधः पात्रं तावज्ज्वाला प्रदीयते ।।

स्वाङ्गशीतं समुद्धृत्य माणिक्याभं हरेद्रसम् ।

तद्रक्तिद्वितयं खादेद्धृतभ्रामरमर्दितम् ।।

सम्पूज्य देवदेवेशं कुष्ठरोगाद्विमुच्यते ।

स्फुटितं गलितं कुष्ठं वातरक्तं भगन्दरम् ।।

नाडीव्रणं व्रणं दुष्टमुपदंशं विचर्चिकाम् ।

नासाऽऽस्य सम्भवान् रोगान् क्षतान् हन्ति सुदारुणाम् ।।

पुण्डरीकं चर्मदलं विस्फोटं मण्डलं तथा ।

Rasendra Chintamani 9/128-133 [1]

Ingredients: Shodhit Hartala – 100 Gm

Equipment required for the process: Earthen sharav, Badari patra, Khalva Yantra (Mortar and Pestle), Weighing machine, Container, Gas Stove, Knife

Procedure

1. 100 gm of Shodhit Haratal was taken in a Sharav and spread uniformly.
2. Another Sharav of same size was kept above the lower Sharav.
3. Then Sandhibandhan was done with Badri kalka to fix the 2 Sharavas.
4. On drying of the Sandhibandhan, the Samput was subjected to Agni.
5. This sharav samputa is kept on fire and heated till the lower sharava turned Arun Varna i.e. red hot.
6. After it Samputa was allowed for self-cooling.
7. On self-cooling, the Samput was opened by removing badari patra kalka sandhibandhan

with the help of knife.

8. The flakes of Rasamanikya are collected from the lower Sharav on scratching.
9. The flakes of Rasamanikya were collected carefully and stored in an air tight container after making powder of them in khalva yantra.

Precautions

1. Small size i.e. Tandulakruti of Shodhit hartala used in the preparation of rasamanikya
2. Constant Agni was maintained during whole procedure.

Figure 3: Preparation of Rasamankiya.

Figure 4: Final Product rasamanikya.

B. Analytical Study[2]

The prepared Rasamanikya was subjected to organoleptic evaluation based on appearance, colour, odour, taste, and touch. Physicochemical parameters including moisture content, total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive, pH, specific gravity, and volatile matter were analysed using standard analytical procedures.

Further analytical characterization was carried out by X-ray fluorescence (XRF) for elemental analysis, X-ray diffraction (XRD) for crystalline characterization, and particle size analysis for determination of particle size distribution and polydispersity index.

C. Experimental Study i.e. In vitro cell line study[3,4,5,6]

The in vitro cytotoxic activity of Rasamanikya was evaluated on A431 skin cancer cell line using MTT assay. Cells were treated with different concentrations of Rasamanikya (1000, 500, 250, 125, and 62.5 µg/mL) and incubated at 37°C in 5% CO_2 atmosphere for 48 hours. After incubation, MTT reagent was added and the formed formazan crystals were dissolved using DMSO. Absorbance was measured using microplate reader, and percentage cell viability along with IC_{50} value was calculated. The assay was performed in triplicate.

Table 1: Details of test compound concentrations.

Sr. No.	Test Compounds	Cell Line	Concentration treated to cells
1	Untreated	A431	No treatment
2	Blank	A431	Only Media without cells
3	Test formulation	A431	5 (1000, 500, 250, 125 and 62.5 µg/mL)

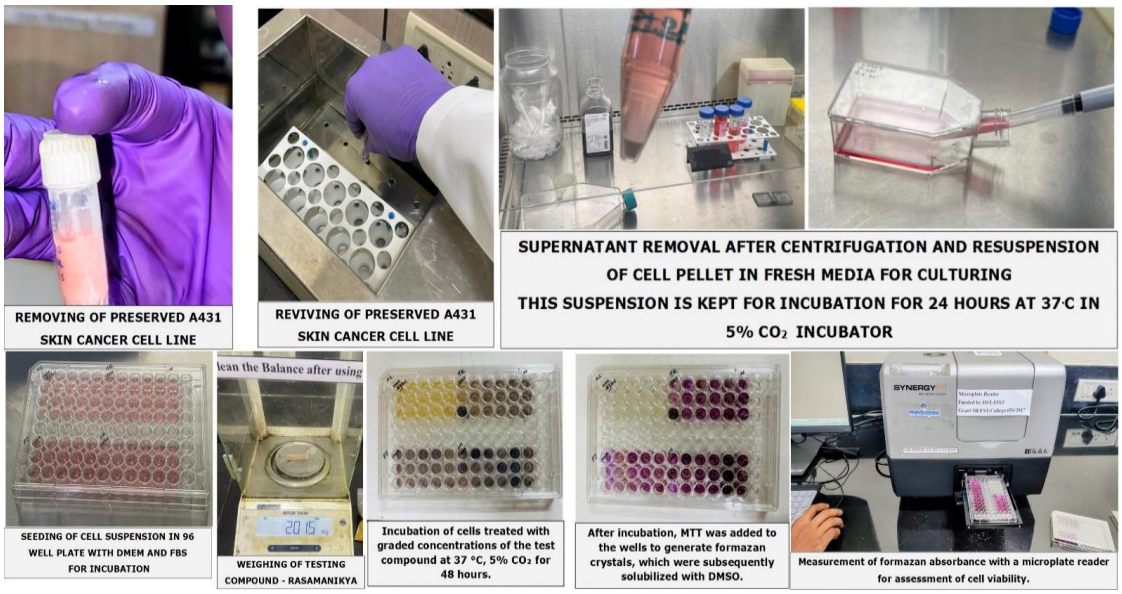


Figure 5 - In vitro cell line study.

RESULTS

The observations and results obtained from the present study, which include the pharmaceutical study of Rasamanikya, the Analytical study, and the Experimental (in vitro) study of the cytotoxic activity of Rasamanikya on A431 skin cancer cell line of Rasamanikya, are presented below under the following headings:

1. Pharmaceutical study result
2. Analytical study result
3. Experimental study result

1. Pharmaceutical study result

The pharmaceutical study was carried out systematically in accordance with the guidelines described in the classical text Rasendrachintamani.

The entire procedure was performed in two main stages:

A. Shodhan of Hartal

(a) Hartal shodhan in kushmand Svarasa

(b) Hartal shodhan in Dadhi

B. Preparation of Rasamanikya

1A. Observations Recorded in the shodhan of Hartal

Table 1: Observation of Hartal shodhan in kushmand Swarasa.

day	date	ashuddh hartal		volume of swaras taken	ph of kushmand swarsa		end date	washing	shodhit hartal	
		colour	weight		before	after			colour	weight
1	30.12.24	dark orange	400 gm	900 ml	5.9	6.3	31.12.24	2	dark orange	404 gm
2	31.12.24	dark orange	404 gm	900 ml	5.7	6.0	01.01.25	2	dark orange	410 gm
3	01.01.25	dark orange	410 gm	900 ml	5.8	6.1	02.01.25	2	golden yellow	410 gm
4	02.01.25	golden yellow	410 gm	900 ml	5.7	6.1	03.01.25	2	golden yellow	407 gm
5	03.01.25	golden yellow	407 gm	900 ml	5.9	6.4	04.01.25	2	golden yellow	410 gm
6	04.01.25	golden yellow	410 gm	900 ml	5.8	6.2	05.01.25	2	golden yellow	412 gm
7	05.01.25	golden yellow	412 gm	900 ml	5.9	6.2	06.01.25	2	golden yellow	415 gm

After 7 days shodhan of hartal in Kushmand Swaras it is washed and dried in sun.

Weight of hartal Before drying in sun is 415 gm and after drying it becomes 397 gm.

From this 5 gm of sample was taken out for analytical study.

Table 2: Observation of Hartal shodhan in Dadhi.

day	date	ashuddh hartal		volume of dadhi taken	ph of dadhi		end date	washing	shodhit hartal	
		colour	weight		before	after			colour	weight
8	06.01.25	golden yellow	392 gm	800 ml	3.4	3.0	07.01.25	2	golden yellow	389 gm
9	07.01.25	golden yellow	389 gm	800 ml	3.6	3.2	08.01.25	2	golden yellow	391 gm
10	08.01.25	golden yellow	391 gm	800 ml	4.1	3.7	09.01.25	2	golden yellow	387 gm
11	09.01.25	golden yellow	387 gm	800 ml	3.8	3.4	10.01.25	2	golden yellow	386 gm
12	10.01.25	golden yellow	386 gm	800 ml	3.9	3.4	11.01.25	2	golden yellow	384 gm
13	11.01.25	golden yellow	384 gm	800 ml	3.7	3.2	12.01.25	2	light yellow	381 gm
14	12.01.25	light yellow	381 gm	800 ml	3.6	3.3	13.01.25	2	light yellow	379 gm

Table 3: Observation of Weight Change in Hartal During Shodhan.

observation of	hartal shodhan in kushmand swaras	shodhit hartal in kushmand swaras after drying in sun	hartal shodhan in dadhi	shodhit hartal in dadhi after drying in sun
initial amount	400 gm	415 gm	392 gm	379 gm
final amount	415 gm	397 gm (5gm sample taken for analytical study)	379 gm	370 gm

After 7 days shodhan of hartal in Dadhi it is washed and dried in sun.

Weight of hartal Before drying in sun is 379 gm and after drying it becomes 370 gm.

1B. Observation of Temperature while Preparation of Rasamanikya

Table 4: Temperature Noted Every 10 Minutes in Preparation of Rasamanikya.

Time	Temperature	Observations
5.20 PM	42 ◦C	
5.30 PM	58 ◦C	
5.40 PM	85 ◦C	
5.50 PM	124 ◦C	
6.00 PM	185 ◦C	
6.10 PM	218 ◦C	Some fumes started coming out from sandhibandhan
6.20 PM	232 ◦C	Lower sharav become red hot

6.30 PM	240 ·C	
6.40 PM	250 ·C	
6.50 PM	226 ·C	
7.00 PM	200 ·C	
7.10 PM	98 ·C	
7.20 PM	41 ·C	

Observations in Short

Total time required for preparation of Rasmanikya is 2 hours.

Temperature required is -250 ^{0}C

Weight of raw product is – 100 gms

Weight of final product is -80 gms

Loss of Wt. during the process is -20 gms

Colour of raw product is – Dark Orange

Colour of final product is - Light Yellowish

2. Analytical Study Result

Table 5: Comparison Of Observation of Organoleptic Parameters.

Observation of Organoleptic parameters	**Raw Hartala**	**Hartala Shodhit in kushmand swaras**	**Hartala Shodhit in Dadhi**	**Rasamanikya**
Appearance	Hard Powdered Crystals	Hard Powdered	Hard Powdered	Fine Powder
Colour	Dark Orange	Dark Brown	Light Brown	Light Yellowish
Odour	Sulphurus	Faint Sulphurus	Faint Sulphurus	Odorless
Taste	Acrid	Slight Acrid	Slightly Acrid	Tasteless
Touch	Rough	Slight Soft	Slightly Soft	powder

Table 6: Comparison Of Observation of Physico-Chemical Parameters.

Observation of Physico-chemical parameters	**Raw Hartala**	**Hartala Shodhit in kushmand swaras**	**Hartala Shodhit in Dadhi**	**Rasamanikya**
Moisture Content	1.9 %	1.1 %	0.90 %	0.16 %
Total Ash (%w/w)	4.11 %	3.95 %	3.54 %	3.16 %
Acid Insoluble Ash (%w/w)	1.24 %	1.68 %	1.59 %	1.96 %
Water soluble extractive (%w/w)	-	-	-	1.46 %
Alcohol soluble extractive (%w/w)	-	-	-	1.55 %
pH	8.9	8.3	8.1	5.6
Specific gravity	1.65	1.69	1.46	0.998
Volatile matter	-	-	-	96.67 %

XRF Analysis

XRF analysis demonstrated arsenic and sulphur as the major constituents of Rasamanikya. The formulation contained arsenic (64.4%) and sulphur (28.3%) as principal elements. Other elements detected included silicon (3.78%), aluminium (2.18%), antimony (1.02%), zinc (0.155%), iron (0.0816%), and nickel (0.0239%).

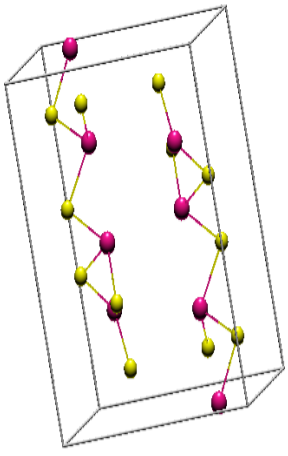
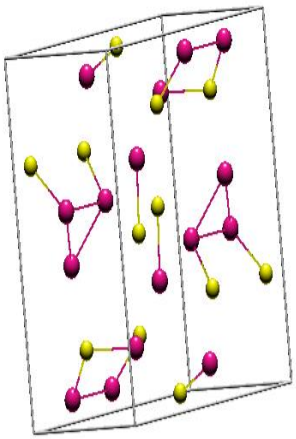
Table 7: Observation of XRF.

Component	Raw Hartala	Hartala Shodhit in kushmand swaras	Hartala Shodhit in Dadhi	Rasamanikya
As	63.9	62.6	64.7	64.4
S	33.9	35.2	34.1	28.3
Si	1.64	0.409	0.352	3.78
Sb	0.487	0.856	0.614	1.02
Fe	0.0378	0.0426	0.0533	0.0816
Zn	0.0263	0.0530	0.156	0.155
Cu	0.0126	0.0145	-	-
Al	-	-	-	2.18
Ni	-	-	-	0.0239
K	-	0.807	-	

XRD Analysis

XRD analysis revealed crystalline transformation during pharmaceutical processing. Raw Hartala was identified as Orpiment (As_2S_3), whereas Rasamanikya was identified as Dimorphite (As_4S_3).

Table 8: Comparison of Observation of XRD.

Raw Hartala	Rasamanikya
Orpiment	Dimorphite
As_2S_3	As_4S_3
	

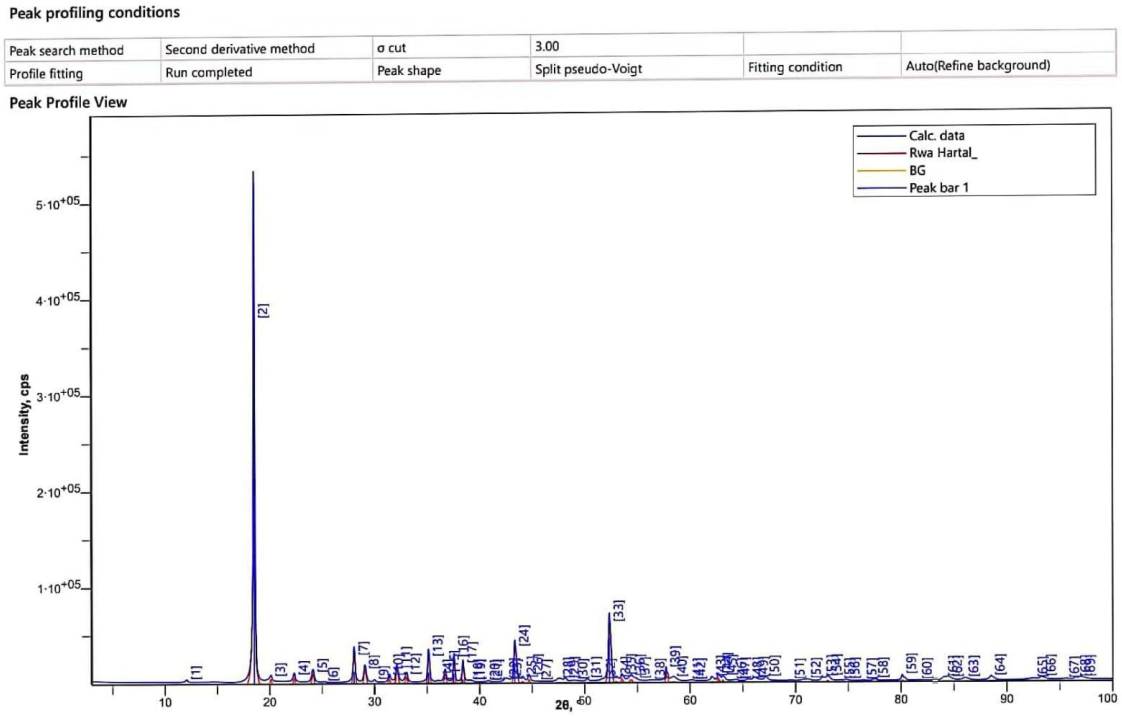


Figure 6: Peak Profile view of XRD of Raw Hartala.

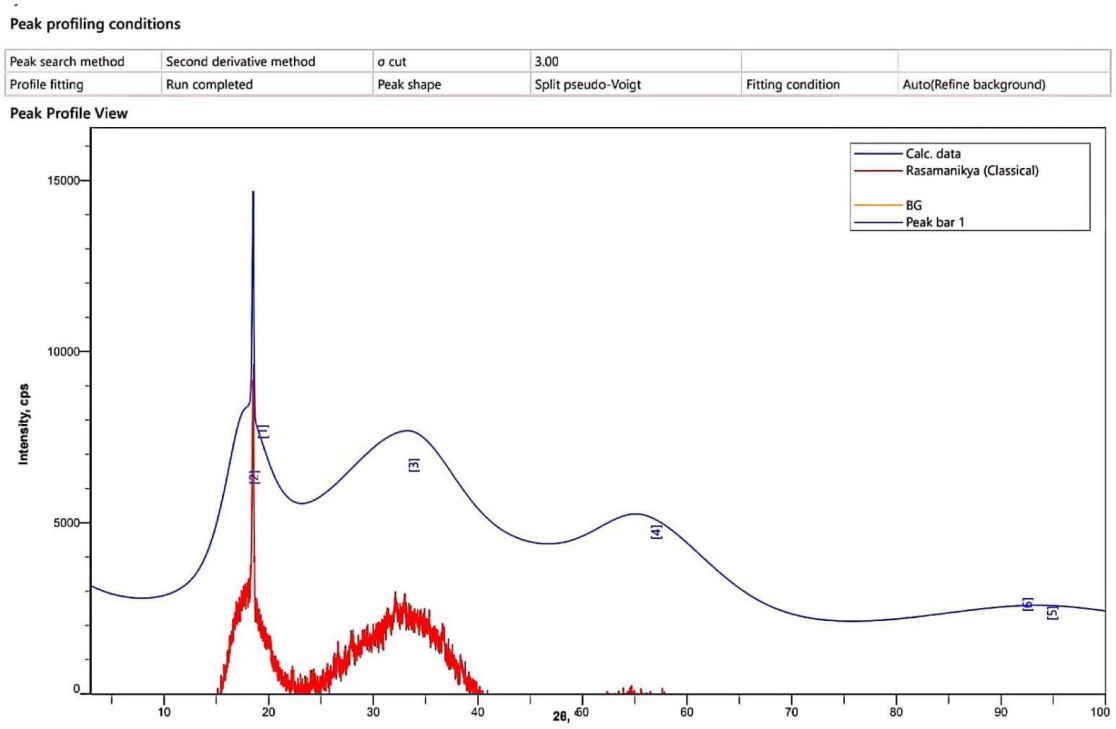


Figure *7*: Peak Profile view of XRD of Rasamanikya.

Particle size analysis

The sample has a primary particle size around 385 nm, with a Z-average of 686 nm, and a moderate PI (0.602), suggesting some distribution spread but overall monodisperse nature.

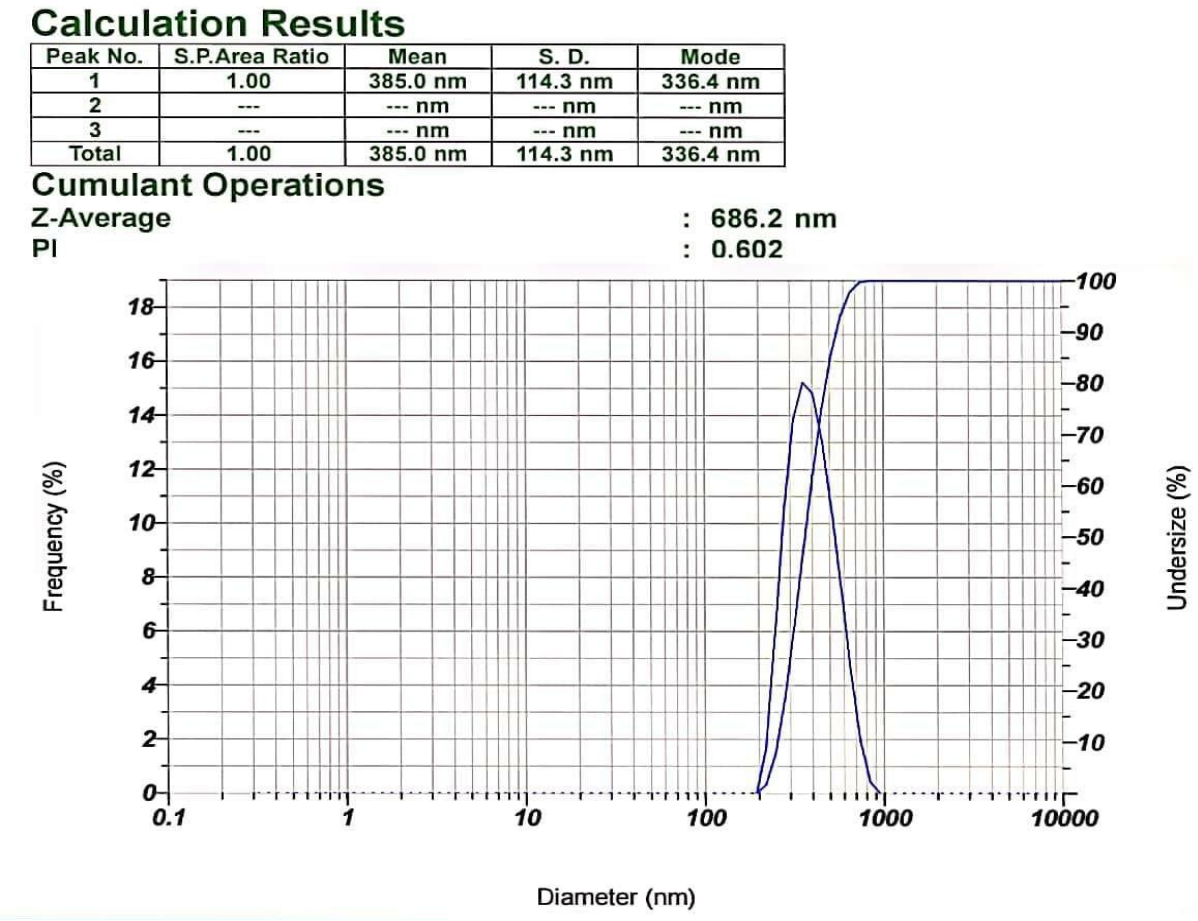


Figure 8: Particle size distribution of Rasamanikya.

3. Experimental Study Result

Cytotoxic Activity of Rasamanikya on A431 Cell Line

The MTT assay was performed in triplicate, and the results were expressed as Mean. Statistical analysis was carried out using GraphPad Prism software to ensure accuracy and reproducibility of the data.

Table 9: Corrected Absorbance After MTT Assay.

Treatments	Conc. (µg/mL)	Abs (n1)	Abs (n2)	Abs (n3)
Test Formulation	1000	0.268	0.483	0.376
Test Formulation	500	0.657	0.594	0.769
Test Formulation	250	1.394	1.032	0.896
Test Formulation	125	0.945	0.923	0.9
Test Formulation	62.5	2.165	1.383	1.419
DMEM+ Cell	-	1.242	1.53	1.631
DMEM	-	0	0	0

The MTT assay was conducted to evaluate the cytotoxicity of the Rasamanikya on the A431 Skin cancer cell line. Cells were treated with varying concentrations of the formulation (1000, 500, 250, 125, and 62.5 µg/mL). A dose-dependent decrease in cell viability was observed with increasing concentrations of the drug. At lower concentrations, only a mild reduction in viability was noted, whereas at higher concentrations, Rasamanikya produced a marked inhibitory effect, reducing viability by more than 50%. This indicates cytotoxic potential of drug.

Table 10: Percentage Cell Viability BY The Treatments.

Treatments	Conc. (µg/mL)	% cell viability (n1)	% cell viability (n2)	% cell viability (n3)
Test Formulation	1000	21.5780998	31.5686275	23.0533402
Test Formulation	500	52.8985507	38.8235294	47.1489884
Test Formulation	250	112.238325	67.4509804	54.9356223
Test Formulation	125	76.0869565	60.3267974	55.1808706
Test Formulation	62.5	174.31562	90.3921569	87.0018394
DMEM+ Cell	-	100	100	100

The **IC_{50} value** was calculated as **197.6 µg/mL** with a 95% confidence interval of 95.54–399.6 µg/mL. The Hill slope was –1.407 (95% CI: –3.168 to –0.5076), indicating a negative slope with progressive inhibition of cell growth. The coefficient of determination (R^2) was 0.5047, which suggests a moderate fit of the experimental data to the dose-response curve.

These findings confirm that Rasamanikya exhibits a dose-dependent cytotoxic effect on A431 cells, with measurable inhibitory activity.

[Inhibitor] vs. normalized response -- Variable slope	
Best-fit values	
IC50	**197.6**
Hill Slope	-1.407
logIC50	2.296
95% CI (profile likelihood)	
IC50	95.54 to 399.6
Hill Slope	-3.168 to -0.5076
logIC50	1.980 to 2.602
Goodness of Fit	
Degrees of Freedom	15
R squared	0.5047
Sum of Squares	13189
Sy.x	29.65
Constraints	
IC50	IC50 > 0
Number of points	
# of X values	18
# Y values analyzed	17

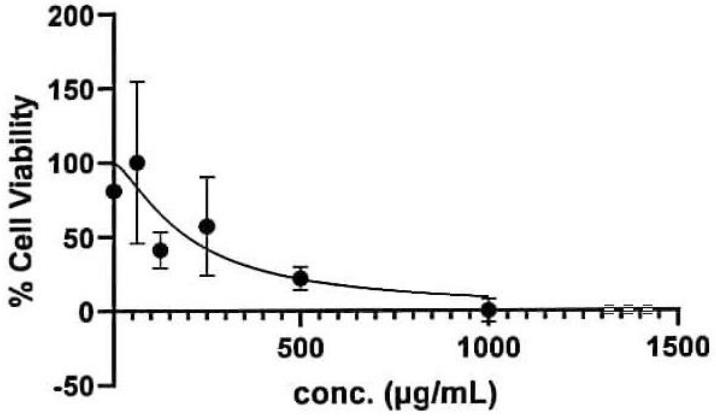


Figure 9: Dose–Response Curve of Rasamanikya on A431 Cell Line.

At lower concentrations, Rasamanikya showed limited effect on cell viability, whereas higher concentrations produced marked cytotoxicity, reducing viability by more than 50%. These results confirm that Rasamanikya exerts dose-dependent cytotoxic activity against A431 skin cancer cells.

DISCUSSION

A. Pharmaceutical study

Shodhana of Hartala was carried out by Kshipta method using Kushmanda Swarasa followed by Dadhi as described in Rasendra Chintamani. The purification process resulted in observable changes in colour and weight. The reduction in weight after drying may be attributed to the removal of adhering media and loss of fine particles during washing and filtration. These observations indicate the effect of the purification process on the physical characteristics of Hartala.

Rasamanikya was prepared by Antardhoom method in closed Sharava at 250°C. A weight loss of 20% was observed during preparation, which may be due to adherence of material to the Sharava and physicochemical changes occurring during heating. The final product obtained was a light yellowish fine powder, indicating successful completion of the pharmaceutical process.

B. Analytical Study

Analytical evaluation plays an important role in the standardization and quality assessment of Rasashastra formulations. The prepared Rasamanikya was found to be a light yellowish, odourless, and tasteless powder. A gradual reduction in pH was observed during

pharmaceutical processing, from 8.9 in Raw Hartala to 8.3 after Shodhana in Kushmanda Swarasa, 8.1 after Shodhana in Dadhi, and finally 5.6 in Rasamanikya. This progressive shift from alkaline to mildly acidic nature indicates the influence of the purification media and thermal processing on the physicochemical characteristics of the drug. The low moisture content (0.16%) indicates minimal water content in the formulation and may reduce susceptibility to microbial contamination during storage.

XRF analysis confirmed arsenic and sulphur as the major constituents of Rasamanikya. The slight increase in silicon content in the final product may be attributed to processing in Sharava during preparation.

XRD analysis demonstrated transformation of Hartala from the Orpiment form (As_2S_3) to the Dimorphite form (As_4S_3) after pharmaceutical processing. This crystalline transformation indicates that the heating process induced structural changes in the raw material and may influence its biological properties.

Particle size analysis revealed an average particle size of approximately 385 nm. The reduced particle size may increase surface area and facilitate interaction with biological systems, which could influence the biological activity of the formulation.

C. Cytotoxic Activity on A431 Cell Line

The MTT assay demonstrated a clear dose-dependent reduction in viability of A431 skin cancer cells following treatment with Rasamanikya. Higher concentrations produced greater inhibition of cell growth, whereas lower concentrations showed comparatively less cytotoxic effect. The IC_{50} value of 197.6 µg/mL indicates measurable cytotoxic activity against the tested cell line.

The observed activity may be associated with the physicochemical characteristics of Rasamanikya, including its elemental composition, crystalline transformation, and reduced particle size. The observed dose-response relationship indicates a concentration-dependent cytotoxic effect of the formulation on A431 cells. However, the exact mechanism responsible for the observed activity was not investigated in the present study and requires further exploration.

The findings of the present study provide preliminary scientific evidence supporting the in

vitro cytotoxic potential of Rasamanikya against A431 skin cancer cells. Further in vivo studies and mechanistic investigations are required to establish its safety profile and therapeutic applicability.

CONCLUSION

The present study successfully established the pharmaceutical and analytical parameters of Rasamanikya prepared according to the method described in Rasendra Chintamani. Shodhana and pharmaceutical processing resulted in significant physicochemical changes in Hartala, as evidenced by alterations in pH, elemental composition, crystalline structure, and particle size. XRD analysis demonstrated the transformation of Orpiment (As_2S_3) into Dimorphite (As_4S_3), while particle size analysis revealed particles in the nanometer range.

The in vitro cytotoxic study on the A431 skin cancer cell line demonstrated a dose-dependent reduction in cell viability, with an IC_{50} value of 197.6 µg/mL. These findings provide preliminary scientific evidence for the cytotoxic potential of Rasamanikya against A431 skin cancer cells. The study highlights the role of classical Ayurvedic pharmaceutical processing in modifying the physicochemical characteristics of Hartala and producing a formulation with measurable biological activity. Further mechanistic studies and in vivo investigations are required to establish its safety profile and therapeutic applicability.

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