

**ANALYTICAL EVALUATION AND IN VITRO CYTOTOXIC ACTIVITY OF  
ARBUDAHARA RASA****Dr. Rubina B. S.<sup>1\*</sup>, Dr. Sangeeta Rao<sup>2</sup>, Dr. Vikram S.<sup>3</sup>, Dr. Yashwant.<sup>4</sup>**

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**ABSTRACT**

**Background:** Arbudahara Rasa is a classical herbo-mineral formulation described in Ayurvedic literature for the management of Arbuda (tumor-like conditions). Scientific validation of such formulations through analytical and biological studies is essential for establishing quality, safety, and therapeutic potential. **Objective:** To evaluate the physicochemical characteristics, instrumental analytical profile, and in vitro cytotoxic activity of Arbudahara Rasa. **Materials and Methods:** Arbudahara Rasa was prepared according to classical Ayurvedic procedures involving Shodhana, Bhavana, and Puta processes. The finished formulation was subjected to organoleptic evaluation, Bhasma Pareeksha, physicochemical analysis including Loss on Drying (LOD), Total Ash, Acid Insoluble Ash, Water Soluble Ash, pH determination, and mercury estimation. Instrumental characterization was performed using Fourier Transform Infrared Spectroscopy (FTIR) and Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP–OES). Cytotoxic activity was assessed against HepG2 human hepatocellular carcinoma cell lines using the MTT assay and compared with Doxorubicin as a standard drug. **Results:** The formulation appeared as a light brown, fine powder without characteristic odour or taste. Classical Bhasma Pareeksha tests such as Rekhapurnatva, Varitaratva, and Unnama were positive. Physicochemical analysis revealed LOD 1.717% w/w, Total Ash 92.00% w/w, Acid Insoluble Ash 55.46% w/w, Water Soluble Ash 10.87% w/w, mercury content 491 ppm, and pH 9.05. FTIR analysis demonstrated the presence of both organic phytoconstituents and inorganic mineral phases, while ICP–OES confirmed mercury incorporation within the formulation. MTT assay showed concentration-dependent cytotoxic activity against HepG2 cells with an IC<sub>50</sub> value of 202µg/mL. Maximum inhibition of 65.76% was observed at 320µg/mL. **Conclusion:** Analytical findings confirmed the physicochemical stability and successful incorporation of mineral and herbal constituents in Arbudahara Rasa. The formulation demonstrated moderate dose-dependent cytotoxic activity against HepG2 cell lines, suggesting potential anticancer properties that warrant further pharmacological and clinical investigation.

**KEYWORDS:** Arbudahara Rasa, FTIR, ICP-OES, MTT Assay, Cytotoxicity.

**INTRODUCTION**

Ayurveda describes Arbuda as a pathological condition characterized by abnormal and localized tissue growth. Various Rasoushadhis have been mentioned in classical texts for the management of such conditions. Arbudahara Rasa is one such herbo-mineral formulation prepared

through specialized pharmaceutical procedures including Shodhana, Bhavana, and Marana. These processes are believed to transform the raw materials into therapeutically potent and biologically acceptable forms.

Although the formulation has been used traditionally, scientific data regarding its physicochemical characteristics and biological activity remain limited. Therefore, the present study was undertaken to evaluate the analytical profile of Arbudahara Rasa and investigate its *in vitro* cytotoxic activity against HepG2 human liver cancer cell lines.

## MATERIALS AND METHODS

### Preparation of Arbudahara Rasa<sup>[1]</sup>

Arbudahara Rasa was prepared according to the classical method described in Ayurvedic literature. Purified Parada and other ingredients were subjected to prescribed pharmaceutical procedures including Shodhana and Bhavana using Tanduliyaka Swarasa, Punarnava Kwatha, Tambula Swarasa, Kumari Swarasa, Bala Moola Kashaya, and Gomutra and subjected to Gorvaraputa.

### Organoleptic Evaluation

The prepared formulation was evaluated for colour, odour, taste, and texture using standard sensory examination methods.

### Bhasma Pareeksha<sup>[2]</sup>

Classical quality control tests including Rekhapurnatva, Varitaratva, and Unnama were performed to assess fineness and suitability of the formulation.

### Physicochemical Analysis<sup>[3]</sup>

The following parameters were determined using standard analytical methods:

- Loss on Drying (LOD)
- Total Ash
- Acid Insoluble Ash
- Water Soluble Ash
- pH
- Mercury content

### FTIR Analysis<sup>[4]</sup>

FTIR spectroscopy was performed to identify functional groups and assess organic-inorganic interactions within the formulation.

### ICP-OES Analysis<sup>[5]</sup>

Mercury concentration was estimated using Inductively Coupled Plasma-Optical Emission Spectroscopy.

### In Vitro Cytotoxicity Study<sup>[6]</sup>

Cytotoxic activity was evaluated against HepG2 human hepatocellular carcinoma cell lines using the MTT assay. Doxorubicin was used as the standard reference drug. Cell viability and percentage inhibition were calculated at various concentrations.

## RESULTS

### Organoleptic Evaluation

Parameter	Observation
Colour	Light Brown
Odour	No characteristic odour
Taste	No characteristic taste
Touch	Smooth, fine powder

### Bhasma Pareeksha

Test	Result
Rekhapurnatva	Positive
Varitaratva	Positive
Unnama	Positive

### Physicochemical Analysis

Parameter	Result
Loss on Drying	1.717% w/w
Total Ash	92.00% w/w
Acid Insoluble Ash	55.46% w/w
Water Soluble Ash	10.87% w/w
Mercury Content	491 ppm
pH	9.05

### FTIR Findings

Major absorption peaks were observed at 3313, 1618, 1433, 1322, 987, 965, 799, 779, 733, 692, 677, 621, 601, 576, 555, 544, 533, 526, and 508  $\text{cm}^{-1}$ . These peaks indicated the presence of hydroxyl, aromatic, amine, carboxyl, and metal-oxygen functional groups.

### ICP-OES Analysis

Mercury concentration in Arbudahara Rasa was found to be 491 ppm.

### MTT Assay

Concentration ( $\mu\text{g/mL}$ )	% Inhibition
10	12.33
20	19.82
40	29.00
80	37.38
160	44.02
320	65.76

**IC<sub>50</sub> of Arbudahara Rasa:** 202  $\mu\text{g/mL}$

**IC<sub>50</sub> of Doxorubicin:** 4.456  $\mu\text{M}$

## DISCUSSION

The organoleptic and classical Bhasma Pareeksha findings confirmed successful pharmaceutical processing of Arbudahara Rasa. The low moisture content observed through LOD analysis indicates adequate drying and enhanced formulation stability. The high total ash value reflects the predominance of inorganic mineral constituents, which is characteristic of Rasoushadhis.

The elevated acid insoluble ash suggests the presence of stable mineral complexes formed during repeated pharmaceutical processing, whereas water soluble ash indicates the availability of soluble inorganic

constituents that may contribute to bioavailability. The alkaline pH observed in the formulation may be attributed to mineral components and Gomutra Bhavana.

FTIR analysis demonstrated the coexistence of herbal phytoconstituents and mineral components. Functional groups corresponding to hydroxyl, aromatic, and amine compounds indicate retention of bioactive phytochemicals from the Bhavana media. Strong metal–oxygen vibrations observed in the fingerprint region suggest stabilization and transformation of mineral constituents.

ICP–OES analysis confirmed the presence of mercury in the finished formulation. The detected mercury concentration indicates successful incorporation of processed Parada following classical purification procedures. The possibility of mercury existing in a transformed and stabilized state is supported by FTIR findings.

The MTT assay demonstrated concentration-dependent cytotoxic activity against HepG2 cells. Cell inhibition increased progressively with increasing concentration, reaching 65.76% at 320 µg/mL. The IC<sub>50</sub> value of 202 µg/mL indicates moderate antiproliferative activity. Although the formulation was less potent than Doxorubicin, microscopic observations revealed reduced cell density and altered morphology in treated cells, supporting its cytotoxic potential. The observed activity may be attributed to synergistic interactions between mineral constituents and phytochemicals incorporated during Bhavana.

## CONCLUSION

The present study established the analytical profile and *in vitro* cytotoxic potential of Arbudahara Rasa. Physicochemical and instrumental analyses confirmed the presence of stable mineral phases along with herbal phytoconstituents. Mercury was successfully incorporated within the formulation following classical pharmaceutical processing. The formulation exhibited moderate dose-dependent cytotoxic activity against HepG2 human liver cancer cells. These findings provide preliminary scientific evidence supporting the traditional therapeutic relevance of Arbudahara Rasa and justify further preclinical and clinical studies to explore its mechanism of action and therapeutic applications.<sup>[7]</sup>

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