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# Pharmaceutical, Physicochemical and Preliminary In Vitro Cytotoxic Evaluation of Mallapushpa Prepared by Gouripashana Satvapatana

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Abstract :

**Background:** Satvapatana is a pharmaceutical procedure described in Rasashastra for separation of the therapeutically intended fraction (Satva) from selected mineral substances. Mallapushpa, prepared through Gouripashana Satvapatana, was subjected in the present study to pharmaceutical, physicochemical, elemental and preliminary in-vitro cytotoxic evaluation.

**Objective**: To prepare Mallapushpa according to the reported classical/procedural source, characterize the obtained preparation using selected physicochemical and elemental parameters, and evaluate its in-vitro cytotoxic effect on A549 and KB-3-1 cancer cell lines.

**Materials and Methods:** Gouripashana and Rakta Kankshi were processed as described in the classical texts using Shodhana, Bhavana with Nimbu Swarasa and Sattvapatana in Damaru Yantra. The preparation was evaluated for organoleptic characters, pH, total ash, loss on drying, loss on ignition, water solubility, particle size and SEM-EDX elemental composition. Cytotoxicity was assessed against A549 and KB-3-1 cells using the reported concentration range, with cisplatin as comparator.

**Results:** The reported preparation yielded 45 g of Mallapushpa from 600 g of combined processed starting materials, corresponding to 7.5% on that stated basis. The preparation was creamish-white, smooth and fine powdered, with pH 4.18, total ash 89.21% w/w, loss on drying 3.8% w/w, loss on ignition 10.79% w/w and particle size reported as 19.7 µm. SEM-EDX reported As 93.2 wt%, Al 6.5 wt% and K 0.3 wt% at the analyzed area. Mallapushpa produced concentration-dependent reduction in viability in A549 and KB-3-1 cells under the reported conditions.

**Conclusion**: Mallapushpa was prepared and showed preliminary concentration-dependent in-vitro cytotoxic activity in the two tested cancer cell lines. The findings do not establish selective anticancer efficacy, clinical effectiveness or

human safety. Verification of raw batch records, complete assay methodology, statistical replication, chemical characterization/speciation and toxicological evaluation is required before therapeutic conclusions can be drawn.

**Keywords:** Mallapushpa; Satvapatana; Gouripashana; Rakta Kankshi; physicochemical characterization; SEM-EDX; A549; KB-3-1;in-vitro cytotoxicity.

## INTRODUCTION

Rasashastra is a branch of Ayurveda concerned with the pharmaceutical processing and therapeutic application of Rasa Dravyas. Procedures such as Shodhana, Marana, Jarana, Amritikarana and Satvapatana are described for processing mineral and metallic substances. Satvapatana refers to separation/extraction of the intended Satva or essence from a suitable Dhatu/Dravya by pharmaceutical procedures.

Gouripashana is described in the Ayurvedic literature among mineral substances and is identified in the present study with arsenic trioxide (As2O3). Because arsenic-containing preparations require particular caution its result interpretation, analytical characterization and safety assessment are essential.

The present work investigates the pharmaceutical preparation of Mallapushpa through Gouripashana Satvapatana, followed by selected physicochemical and elemental characterization and preliminary in-vitro cytotoxic evaluation against A549 and KB-3-1 cancer cell lines. The study is restricted to the experimental observations reported and does not infer clinical anticancer efficacy or safety.

## NEED OF THE STUDY.

### AIMS AND OBJECTIVES

**Aim**: To prepare Mallapushpa through Gouripashana Satvapatana and evaluate its selected pharmaceutical, physicochemical, elemental and preliminary in-vitro cytotoxic characteristics.

To study the pharmaceutical preparation of Mallapushpa.

To document its organoleptic characteristics.

To evaluate selected physicochemical and elemental parameters.

To evaluate the in-vitro cytotoxic effect of Mallapushpa on A549 and KB-3-1 cancer cell lines under the specified experimental conditions.

### MATERIALS AND METHODS

Study setting and authentication

The pharmaceutical work was reported to have been performed in the Post-Graduate Department of Rasashastra and Bhaishajya Kalpana, Ayurveda Medical College, Hubballi, Karnataka, India. The ingredients were procured from the market and authenticated by departmental experts as stated in the original classics.

Materials

**Table 1. Ingredients reported in the original manuscript.**

| S. No. | Ingredient | Chemical formula / botanical source | Proportion | Quantity reported |
|---|---|---|---|---|
| 1 | Shodhita Gouripashana | As2O3 | 1 part | 400 g |
| 2 | Shodhita Rakta Kankshi | KAl(SO4)2·12H2O | 1 part | 600 g |
| 3 | Nimbu Swarasa | Citrus medica | ¼ part | 120 mL |

Shodhana of Gouripashana and Rakta Kankshi

| Raw material | Method adopted | Duration | Number of times |
|---|---|---|---|
| Grahya Rakta Kankshi | Nirjalikarana | 1 hr 30 min | 1 |
| Grahya Gouripashana | Swedan in godugdha | 3 hrs | 1 |

**Table 2. Reported observations during Shodhana.**

| Material | Before Shodhana | After Shodhana | Loss | Time |
|---|---|---|---|---|
| Rakta Kankshi | 600 g | 320 g | 280 g | 1 h 30 min |
| Gouripashana | 400 g | 300 g | 100 g | 3 h |

Preparation of Nimbu Swarasa

Approximately 80 ripened Nimbu fruits were reportedly cut into two pieces and juice was extracted by squeezing.

Bhavana of Gouripashana with Nimbu Swarasa

300 g of Shodhita Gouripashana, with 120 mL Nimbu Swarasa, was taken a post-Bhavana weight of 330 g and a total reported duration was 8 h.

**Table 3. Reported Bhavana observations.**

| Shodhita Gouripashana | Nimbu Swarasa | Weight after Bhavana | Reported duration |
|---|---|---|---|
| 300 g | 120 mL | 330 g | 8 h |

Sattvapatana / Preparation of Mallapushpa

The preparation of Mallapushpa describes mixing of Bhavita Gouripashana with Shodhita Rakta Kankshi until a homogeneous mixture was obtained. Two earthen pots were prepared with five layers of Multani-mitti-smeared cloth, dried, filled/arranged as Damaru Yantra, homogeneous mixture was placed in lower pot sealed by Sandhibandhana and heated gradually for 6 hours. The upper pot was cooled with an ice-water-moistened cloth to facilitate condensation. After self-cooling, the deposited whitish material was collected as Mallapushpa.

Recovery and mass balance

**Table 5. Reported recovery of Mallapushpa.**

| Fraction | Recovered mass |
|---|---|
| Mallapushpa – upper pot | 30 g |
| Mallapushpa – lower pot | 15 g |
| Total Mallapushpa | 45 g |
| Initial combined mass stated for final process | 600 g |
| Reported yield | 7.5% |

On the stated basis, the reported yield is calculated as: Yield (%) = (total Mallapushpa recovered / starting mass) × 100 = (45 g / 600 g) × 100 = 7.5%.

Physicochemical and organoleptic evaluation

Organoleptic observations were recorded for colour, odour, touch, taste and texture.

**Table 6. Organoleptic characteristics of Mallapushpa.**

| Parameter | Reported result |
|---|---|
| Colour | Creamish white |
| Odour | Characteristic |
| Touch | Smooth |
| Taste | Sour and tangy |
| Texture | Fine powder |

Physicochemical analysis

**Table 7. Reported physicochemical characteristics.**

| Parameter | Reported result |
|---|---|
| Ph | 4.18 |
| Total ash | 89.21% w/w |
| Loss on drying | 3.8% w/w |
| Water solubility | Partially soluble |
| Loss on ignition | 10.79% w/w |
| Particle size | 19.7 µm (method/details to be verified) |

Particle-size analysis

The original manuscript reports a particle size of 19.7 µm.

SEM-EDX analysis

SEM-EDX was reported to show As, Al and K in the analyzed Mallapushpa sample.

**Table 8. Reported SEM-EDX elemental composition.**

| Element | Weight % | Atomic % |
|---|---|---|
| Al | 6.5 | 16.2 |
| K | 0.3 | 1.9 |
| As | 93.2 | 81.2 |

These EDX results demonstrate the presence and relative abundance of As, Al and K at the analyzed area. EDX alone does not establish oxidation state, molecular speciation or crystalline phase.

Cell culture and cytotoxicity assay

Method adopted :- MTT Assay

Cytotoxicity results

**Table 9. Reported cytotoxicity data and comparator IC50 values.**

| Cell line | Mallapushpa concentration (µg/mL) | Cell viability (%) |
|---|---|---|
| A549 | 500 | 40.92 |
| A549 | 250 | 45.81 |
| A549 | 125 | 52.26 |
| A549 | 62.5 | 60.09 |
| A549 | 31.2 | 70.69 |
| A549 | Reported IC50 | 181.27 µg/mL |
| KB-3-1 | 500 | 37.95 |
| KB-3-1 | 250 | 42.07 |
| KB-3-1 | 125 | 47.47 |
| KB-3-1 | 62.5 | 53.37 |
| KB-3-1 | 31.2 | 68.53 |
| KB-3-1 | Reported IC50 | 132.2 µg/mL* |
| A549 – cisplatin | IC50 | 5.20 µg/mL* |
| KB-3-1 – cisplatin | IC50 | 6.63 µg/mL* |

Normal-cell selectivity

No normal/non-malignant cell line was reported in the present study. Therefore, selective cytotoxicity and therapeutic index were not evaluated.

Classical literature information

The formulation included a statement that Mallapushpa was administered at approximately rice-grain-size dose with Shunti Rasa and mentioned indications such as Shwasa, Kasa, Jwara, Kushta and Rakta Vikara, with avoidance in Pitta-prakriti individuals.

## DISCUSSION

The Sattvapatana process of Bhavita Gouripashana and Shodhita Raktakankshi was carried out in Damru Yantra. Bhavana with Nimbuka swarasa may have facilitated surface-level demineralization and increased the porosity and heat-reactivity of Gouripashana, thereby promoting an effective separation of its sattva. The acidic constituents of Nimbuka swarasa are likely to have weakened the surface mineral matrix and facilitated subsequent thermal processing.

Shodhita Raktakankshi acted as a Sahakari dravya during Sattvapatana. Its presence helped to maintain a suitable thermal environment and supported the separation of sattva during gradual heating. The closed system of Damru Yantra minimized the loss of volatile constituents, provided controlled heating, and facilitated condensation and collection of the liberated material.

During gradual heating, differences in fusibility, density and thermal behaviour of the components facilitated phase separation. The residual material remained as kitta, while the desired sattva was deposited in the collection chamber. The acid–salt interaction between Nimbuka swarasa and Raktakankshi may have further weakened the mineral matrix and enhanced the efficiency of Sattvapatana.

In the present study, 600 g of Bhavita Gouripashana and Shodhita Raktakankshi yielded 45 g of Mallapushpa sattva, corresponding to a yield of 7.5%. Of the total yield, 30 g was deposited in the lower pot and 15 g in the upper pot. The greater deposition in the lower pot may be attributed to the thermal behaviour, condensation and downward settling of volatile arsenic-containing constituents during the heating process. The obtained sattva is free from excessive kitta this indicates effective completion of the Sattvapatana samskara.

**Discussion on Analytical study**

pH: Mallapushpa showed a pH of 4.18, indicating mildly acidic nature, which may be related to its acidic mineral constituents.

Total Ash: The total ash was 89.21%, confirming the predominantly inorganic/mineral nature of Mallapushpa.

Loss on Drying: LOD at 110°C was 3.8%, indicating low moisture content and satisfactory drying and stability of the preparation.

Loss on Ignition: LOI was 10.79%, suggesting the presence of heat-sensitive and volatile constituents along with stable inorganic matter.

Solubility: Mallapushpa was partially soluble in water, indicating the presence of ionizable or water-dispersible mineral constituents.

Particle Size: The average particle size was 19.7 µm. The fine particle size provides greater surface area and may facilitate better dispersion and interaction with the surrounding medium.

SEM–EDX: SEM–EDX demonstrated As, Al and K as the major elements. Their approximate composition was As 93.2%, Al 6.5% and K 0.3%, confirming the arsenic-rich mineral composition of Mallapushpa.

**Discussion on cell line study**

Cell-line Study: Mallapushpa showed a dose-dependent cytotoxic/lysis effect on both lung cancer (A549) and oral cancer (KB) cell lines.

A549: Maximum lysis was observed at 500 µg/mL (59.08%), followed by 250 µg/mL (54.19%). $IC_{50}$ = 181.27 µg/mL.

KB: Maximum lysis was observed at 500 µg/mL (62.05%), followed by 250 µg/mL (57.93%). $IC_{50}$ = 132.2 µg/mL.

Overall, Mallapushpa demonstrated concentration-dependent cytotoxic activity, shows comparatively greater activity against the oral cancer (KB) cell line.

## CONCLUSION

Mallapushpa was prepared by the reported Gouripashana Satvapatana procedure and characterized using selected organoleptic, physicochemical and elemental parameters. Under the reported experimental conditions, Mallapushpa showed concentration-dependent reduction in viability of A549 and KB-3-1 cancer cells. These findings support preliminary in-vitro cytotoxic activity under the tested conditions, but they do not establish selective anticancer efficacy, clinical effectiveness, therapeutic superiority or human safety. Verification of the original pharmaceutical records and cytotoxicity calculations, together with appropriate statistical replication, normal-cell comparison, expanded chemical characterization, mechanistic studies and toxicological/in-vivo evaluation, is required before therapeutic conclusions can be drawn.

## FUTURE SCOPE

- Verification and standardization of the pharmaceutical batch and complete mass balance.
- Expanded chemical characterization including phase/speciation studies where feasible.
- Validated particle-size distribution analysis.
- Repeated cytotoxicity experiments with appropriate biological and technical replicates and statistical analysis.
- Evaluation against an appropriate normal/non-malignant cell line.
- Mechanistic studies to investigate the basis of observed cytotoxicity.
- Toxicological and in-vivo pharmacological evaluation before consideration of clinical research.

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## ANNEXURES



SWEDANA OF GOURIPASHANA



SHODHITA GOURIPASHANA



BHARJANA OF RAKTA KANKSHI



CRYSTALLISATION OF RAKTA KANKSHI



SHODHITA RAKTA KANKSHI



NIMBU SWARASA



**BHAVANA OF SHODHIT SOMALA WITH NIMBU SWARASA**



**BHAVITA GOURIPASHANA**



**MIXING OF BHAVITA SOMALA WITH SHODHITA LALPHITKARI**



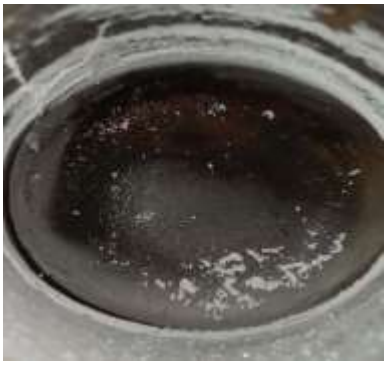
**FILLING OF MIXTURE IN LOWER POT**



**PREPARATION OF DAMRU YANTRA WITH MIXTURE**



**AGNISAMSKARA**



**MALLAPUSHPA AT LOWER POT**



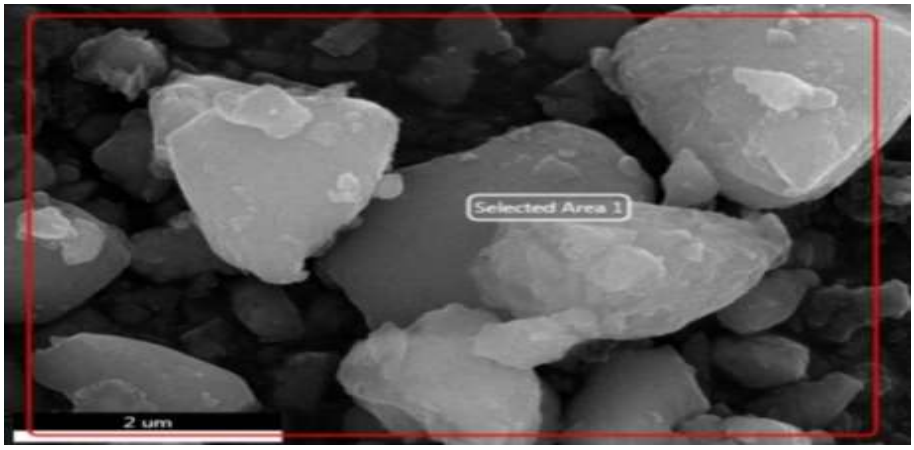
**MALLAPUSHPA AT UPPER POT**



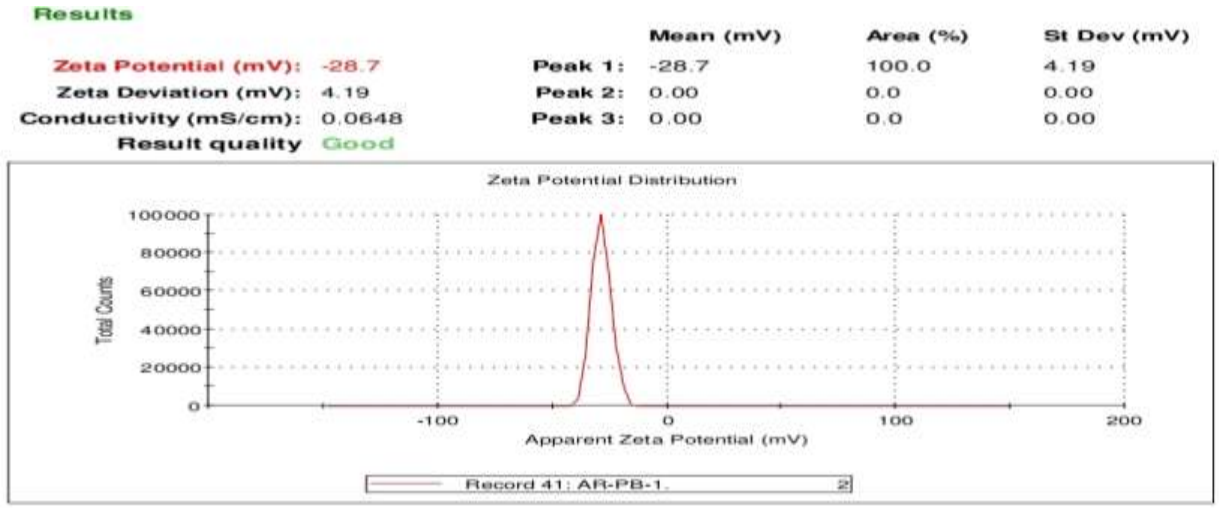
**MALLAPUSHPA**

## ANALYTICAL STUDY

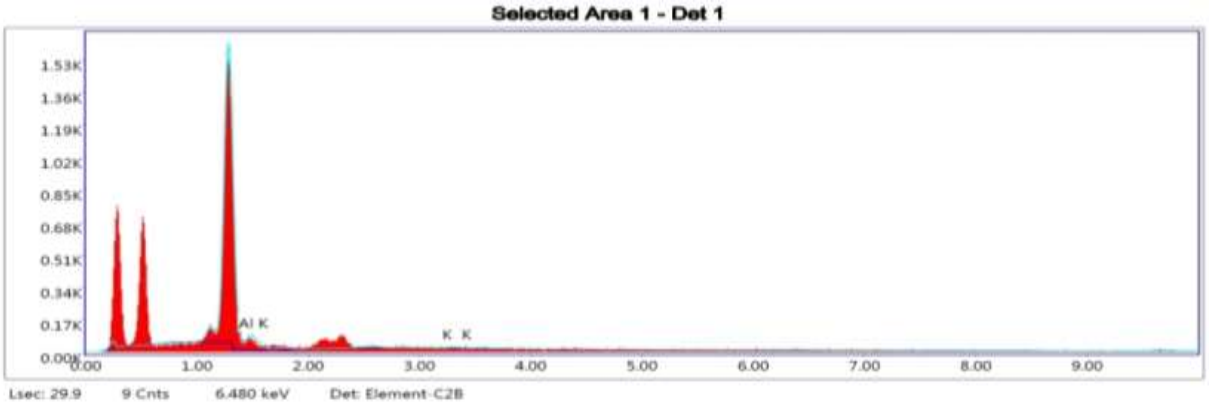
### SEM – EDAX of Mallapushpa




**Graph No 01 . Graph Showing Zeta potential results of Mallapushpa.**




**Graph No 02. Showing results of SEM – EDAX of Mallapushpa.**




**eZAF Smart Quant Results**

| Element | Weight % | Atomic % | Net Int. | Error % | Kratio | Z | A | F |
|---|---|---|---|---|---|---|---|---|
| AlK | 6.5 | 16.2 | 20.7 | 17.8 | 0.0171 | 1.2171 | 0.2159 | 1.0004 |
| K K | 0.3 | 0.5 | 1.9 | 77.5 | 0.0027 | 1.1652 | 0.7394 | 1.0114 |
| AsK | 93.2 | 83.3 | 81.2 | 6.0 | 0.9152 | 0.9801 | 1.0005 | 1.0016 |

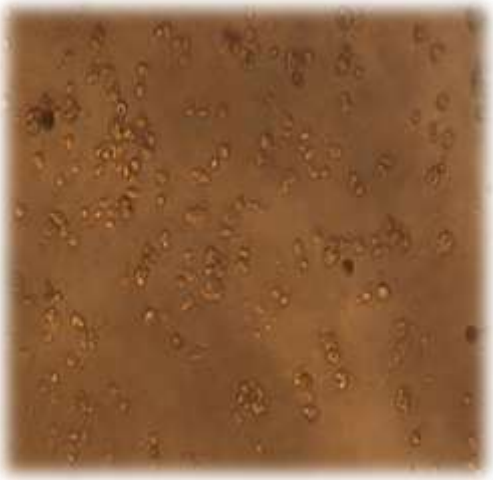
## 4.CELL CULTURE STUDY

**Fig No .04. Experimental study on Mallapushpa at specific concentrations of 500ug/ml and 250ug/ml of A549 Cell and KB Cell**

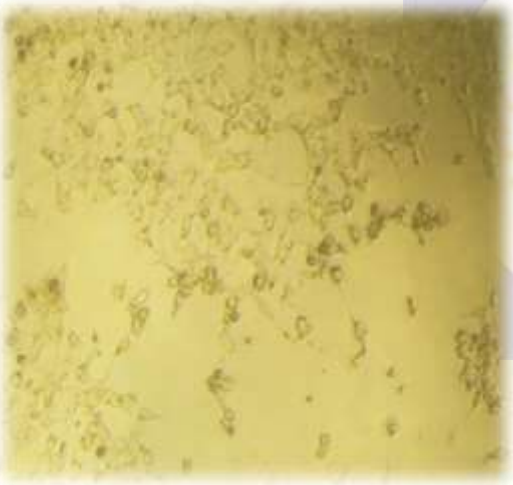
**A549 CELL (500ug/ml)**



**A549 CELL (250ug/ml).**



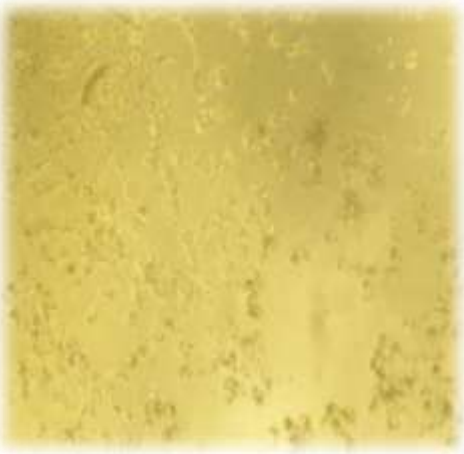
**KB CELL (250ug/ml)**



**KB CELL (500ug/ml)**



**NC A549**



**NC KB**

