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Original Article

In vitro Evaluation of the Anti-Carcinogenic Activity of *Swarnavari Bhasma*: An Ayurvedic Herbo-Mineral Formulation

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ABSTRACT

Background: Cancer remains a formidable global health challenge, and the severe side effects and high costs of conventional treatments like chemotherapy necessitate the exploration of safer, more accessible therapeutic alternatives. Ayurveda, the traditional Indian system of medicine, offers a rich repository of herbo-mineral formulations with potential anticancer properties. This study investigates *Swarnavari Bhasma*, a novel Ayurvedic formulation, for its anti-carcinogenic potential. **Aim:** The primary aim of this study was to prepare and standardize *Swarnavari Bhasma* and to assess its *in vitro* cytotoxic activity against a panel of human cancer cell lines. **Materials and Methods:** *Swarnavari Bhasma* was prepared using traditional Ayurvedic *Rasashastra* methods, including *Shodhana* (purification) and *Marana* (incineration) of its core mineral components, *Swarna Makshika* (*Copper Pyrite*) and *Kapardika* (*Calcium carbonate*). The final formulation was created by integrating these *Bhasmas* with *Haridra* (*Curcuma longa*) and processing them with decoctions of *Triphala, Pippali* (*Piper longum*), and a *Vincristine* solution. The product was characterized using classical Ayurvedic tests (*Bhasma Pariksha*) and modern instrumental techniques including Field Emission Scanning Electron Microscopy (FESEM), X-ray Diffraction (XRD), Particle Size Analysis and Fourier Transform Infrared Spectroscopy (FTIR). The anti-carcinogenic activity was evaluated using the MTT cytotoxicity assay on four human cancer cell lines: MCF-7 (breast), A549 (lung), Hep G2 (liver), and PC-3 (prostate). 5-Fluorouracil (5-FU) was used as the standard positive control. **Results:** *Swarnavari Bhasma* demonstrated significant, dose-dependent cytotoxic activity against all four tested cell lines. The formulation showed the highest efficacy on the MCF-7 breast cancer cell line (IC_{50} = 65.14 µL/mL) and the lowest efficacy against the PC-3 prostate cancer cell line (IC_{50} = 121.28 µL/mL). Analytical characterization confirmed the final product as a highly crystalline, multi-phase herbo-mineral composite with a unique porous, quasi-spherical micro-aggregate morphology and a significant nanoparticle population (mean size ~131.8 nm). **Conclusion:** The study successfully demonstrates that *Swarnavari Bhasma*, prepared and standardized through a combination of classical and modern methods, possesses significant *in vitro* anti-carcinogenic properties. Its multi-targeted nature, derived from the synergy of its diverse components, makes it a promising candidate for further development as an integrative therapy in oncology.

Keywords: Anticancer, Ayurveda, Cytotoxicity, Herbo-mineral formulation, MTT Assay, *Swarnavari Bhasma*.

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INTRODUCTION

Cancer, a multifactorial disease characterized by uncontrolled cell growth, is a leading cause of mortality worldwide. Projections indicate a substantial rise in cancer incidence in India, underscoring the urgent need for novel therapeutic strategies (Aggarwal *et al.*, 2025). While conventional treatments such as chemotherapy and radiation have improved survival rates, they are frequently associated with debilitating side effects, high costs, and the development of drug resistance, limiting their overall effectiveness (Anand *et al.*, 2023; Zafar *et al.*, 2025). This has spurred interest in exploring alternative and integrative treatment options, including those from traditional systems of medicine like Ayurveda.

Ayurveda offers a holistic approach to health, viewing cancer, or *Arbuda*, as a disease arising from an imbalance of the three fundamental biological energies (*Tridoshas*: *Vata, Pitta*, and *Kapha*) that corrupt the body's tissues (*Dhatus*), particularly

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muscle (*Mamsa*) and blood (*Rakta*) (AYUSH Cancer Conclave 2019 Accepted Posters Papers with Abstracts 2019; Prajapati *et al*., 2024). *Rasashastra*, the branch of Ayurvedic pharmaceutics dealing with herbo-mineral preparations, utilizes specific processing techniques to enhance the therapeutic properties of metals and minerals while minimizing toxicity (Savrikar and Ravishankar 2011; Wijenayake *et al*., 2014).

This study focuses on *Swarnavari Bhasma*, a novel herbo-mineral formulation designed based on Ayurvedic principles. It utilizes *Swarna Makshika* (Chalcopyrite) and *Varatika* or *Kapardika* (Cowrie shell) as affordable and effective substitutes (*Abhav Pratinidhi Dravya*) for the traditionally used but costly *Swarna* (Gold) and *Vajra* (Diamond) *Bhasmas* (Sri Govinda Das. Edited by Shri Bramhashankar Mishra. "Bhaishajya Ratnavali". 1st ed. Varanasi; Chaukhambha Sanskrit Bhavan; 2006. Pp.80. n.d.; Vaidya Lakshmipati shashtri, Yogaratnakara ed. With 'Vidyotini' Hindi commentary by Bhimashankar Shastri published by chaukhamba prakashan, Varanasi, reprint-2010-Abhavavarga-verse no.38-page no.173 n.d.) These mineral bases are combined with a synergistic blend of potent herbs. *Haridra* (*Curcuma longa*), known for its active compound curcumin (Fuloria *et al*., 2022), *Triphala*, a classic rejuvenating antioxidant formulation (Bairwa *et al*., 2025) and *Pippali* (*Piper longum*), a well-known bio-enhancer (Unnikrishnan Meenakshi *et al*., 2024). The formulation is further processed with a Vincristine solution, integrating a known chemotherapeutic agent into the traditional matrix (Garg *et al*., 2024).

The objective of this research was to prepare *Swarnavari Bhasma* according to classical Ayurvedic procedures, characterize its physicochemical and structural properties using modern analytical techniques, and evaluate its *in vitro* anti-carcinogenic activity against a panel of human cancer cell lines (Figures 1-9).

MATERIALS AND METHODS

Preparation of *Swarnavari Bhasma*

The formulation was prepared in a multi-step process involving the preparation of intermediate components followed by their final integration. All raw drugs were authenticated prior to use.

The Raw *Swarna Makshika* (SM) first underwent *Shodhana* (purification) via *Nirvapa*, where it was repeatedly heated to a red-hot state and quenched in a freshly prepared *Triphala* decoction (*kwatha*). Subsequently, the purified SM was subjected to *Marana* (incineration) for 12 times by triturating it with purified *Gandhaka* (sulphur) and *Nimbu Swarasa* (lemon juice), forming pellets, and heating them in an Electric Muffle Furnace at 650°C until a fine reddish-brown *Bhasma* was obtained (Pathiraja *et al*., 2020).

The Raw *Kapardika* (cowrie shells) were purified (*Shodhana*) by the *Swedana* (steaming) method in *Kanji* (sour gruel) using a *Dolayantra* for 3 hr. The purified shells were then incinerated (*Marana*) 3 times using a classical *GajaPuta* to obtain a fine, white *Bhasma* (Rasheed and Shivashankar 2017).

The prepared *Swarnamakshik Bhasma* (40 g), *Kapardika Bhasma* (40 g), and *Haridra Churna* (20 g) were combined. This mixture underwent three successive cycles of *Bhavana* (levigation), where it was triturated with *Triphala kwath, Pippali Phanta* (hot infusion), and a Vincristine solution, respectively, until completely dry after each cycle.

Analytical Characterization

The final product and its intermediates were analyzed using both classical and modern methods.

The prepared *Bhasmas* were evaluated for classical quality parameters, including *Varitara* (floating on water), *Rekhapurnatva* (fineness to enter fingerprint lines), and *Slakshnatva* (smoothness) (Mohaptra and Jha 2010; Pal *et al*., 2014).

Standard parameters such as Loss on Drying (LOD), Total Ash, Acid-Insoluble Ash, and pH were determined as per the Ayurvedic Pharmacopoeia of India (Mangal *et al*., 2023).

The final *Swarnavari Bhasma* was characterized using Particle Size Analysis (Dynamic Light Scattering), Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Field Emission Scanning Electron Microscopy (FESEM) to determine its particle size distribution, functional groups, crystalline structure, and surface morphology.

Cell Lines and Culture

Four human cancer cell lines were obtained from the National Center for Cell Sciences (NCCS), Pune-A549 (lung carcinoma), MCF-7 (breast adenocarcinoma), Hep G2 (liver carcinoma), and PC-3 (prostate adenocarcinoma). Each cell line was cultured in its specific growth medium supplemented with 10% Fetal Bovine Serum (FBS) and maintained in a humidified incubator at 37°C with 5% CO_2 to ensure optimal growth conditions.

In vitro Cytotoxicity Assay (MTT)

The cytotoxic potential of the formulation was evaluated using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Cells were seeded in 96-well plates at a density of 1×10^4 cells per well and allowed to adhere for 24 hr. Subsequently, they were exposed to different concentrations of *Swarnavari Bhasma* (10, 40, and 100 µL/mL) and the standard reference drug 5-Fluorouracil (10, 40, and 100 µg/mL) for another 24 hr. After incubation, MTT reagent was added and plates were further incubated for 4 hr to enable viable cells to reduce MTT into insoluble purple formazan crystals. These crystals were solubilized using DMSO, and the absorbance was recorded at 570 nm to determine cell viability (Freimoser *et al*., 1999; Ghasemi *et al*., 2021; Tonder *et al*., 2015).

Statistical Analysis

Experiments were performed in triplicate. Cell viability was calculated as a percentage of the untreated control. Cell viability was expressed as a percentage relative to the untreated control group. The Half-Maximal Inhibitory Concentration (IC_{50}), representing the concentration of the sample required to inhibit 50% of cell proliferation, was derived from the corresponding dose response curves.

RESULTS

Pharmaceutical Preparation and Standardization

The traditional pharmaceutical processes successfully transformed the raw minerals into fine *Bhasmas*. The *Shodhana* of *Swarna Makshika* resulted in a color change from brassy golden-yellow to blackish, indicating successful purification. The final *Swarnavari Bhasma* was a homogenous, brown powder with a characteristic bitter, astringent taste. The prepared *Bhasmas* passed all classical *Bhasma Pariksha* tests, confirming their fine particle size and proper preparation (e.g., *Varitara, Rekhapurnatva*).

Physicochemical and Instrumental Characterization

Physicochemical analysis showed low moisture content (LOD=2.1%) and high purity (Acid-Insoluble Ash=0.2%). A key finding was the high alkalinity of the intermediate *Kapardika Bhasma* (pH 12.1).

FESEM images revealed a unique morphology of porous, quasi-spherical micro-aggregates formed by the sintering of smaller, irregular particles. Particle size analysis confirmed a bimodal distribution with a significant nanoparticle population (mean size 131.8 nm) and a larger microparticle population (mean size 6.67 µm).

XRD analysis confirmed the final product was a highly crystalline, multi-phase composite. The dominant phases were identified as Calcite from *Kapardika* and Chalcopyrite from *Swarna Makshika*, validating the chemical transformation during processing.

The FTIR spectrum provided a chemical fingerprint confirming the integration of all components. Key peaks corresponded to carbonate ions from *Kapardika Bhasma* (1407.38 cm^{-1}), hydroxyl groups from the herbal ingredients (3358.72 cm^{-1}), and a distinct ester carbonyl peak (1742.89 cm^{-1}) attributed to the Vincristine used in processing.

In vitro Cytotoxic Activity

Swarnavari Bhasma exhibited a clear dose-dependent cytotoxic effect on all four cancer cell lines, as evidenced by the detailed absorbance data from the MTT assay. As the concentration of the *Bhasma* increased, the mean absorbance, which corresponds to metabolic activity, decreased, indicating a reduction in cell viability. The formulation was most effective against the MCF-7 (breast cancer) cell line and least effective against the PC-3 (prostate cancer) cell line. While the standard chemotherapeutic agent 5-FU was significantly more potent in all cases, the intrinsic bioactivity of *Swarnavari Bhasma* was clearly established. The calculated IC_{50} values are summarized in Table 1.

The bar chart illustrates the differential sensitivity of the cell lines to the formulation, with MCF-7 being the most sensitive. Note the logarithmic scale on the Y-axis to accommodate the large difference in potency between the *Bhasma* and 5-FU.

Table 1: Summary of IC_{50} Values against Human Cancer Cell Lines.

Cell Line	Cancer Type	IC_{50} of Swarna *Vari Bhasma* (µL/mL)	IC_{50} of 5-Fluorouracil (µg/mL)
MCF-7	Breast	65.14	6.00
Hep G2	Liver	70.96	14.22
A549	Lung	92.14	25.27
PC-3	Prostate	121.28	9.75

Table 2: Raw Absorbance and Viability Data for A549 (Lung Cancer) Cells.

Treatment Group	Concentration	Absorbance (570 nm) Replicates	Mean Absorbance	% Cell Viability	% Inhibition
Control	0 µL/mL	2.226	2.225	2.224	2.225
Swarnavari Bhasma	10 µL/mL	1.896	1.895	1.892	1.894
	40 µL/mL	1.753	1.756	1.759	1.756
	100 µL/mL	0.998	0.997	0.994	0.996
5-Fluorouracil (Std.)	10 µg/mL	1.263	1.264	1.264	1.263
	40 µg/mL	0.962	0.963	0.961	0.962
	100 µg/mL	0.426	0.423	0.421	0.423

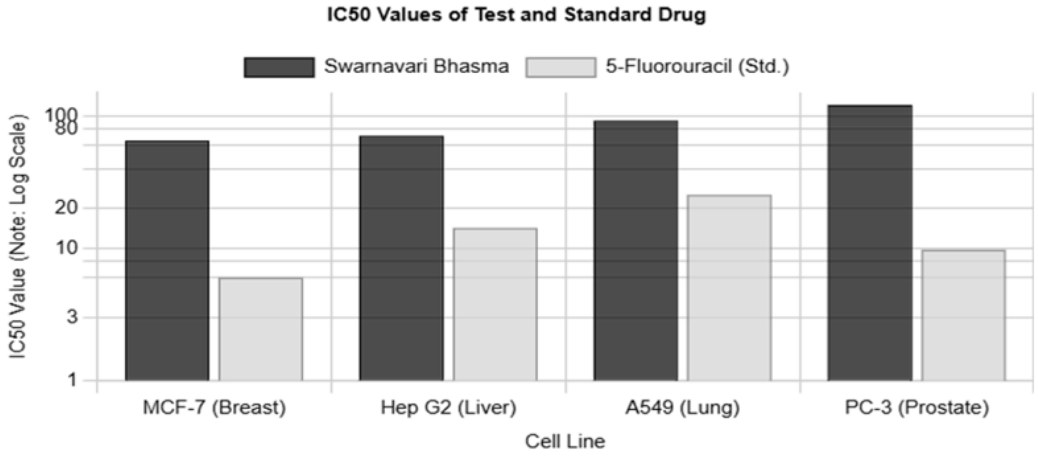

Figure 1: Comparative IC_{50} values of Swarnavari Bhasma and 5-FU across all tested cell lines.

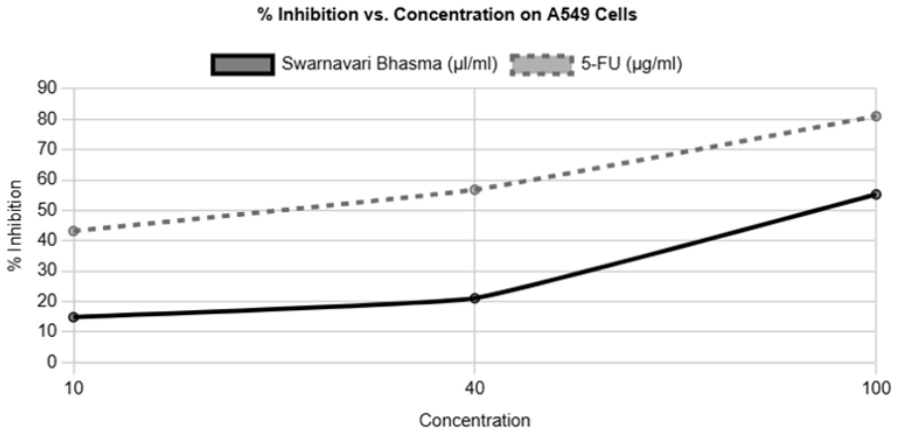

Figure 2: Dose-response curves for A549 cells. The graph shows the percentage of cell viability as a function of the concentration of *Swarnavari Bhasma* and 5-FU.

Table 3: Raw Absorbance and Viability Data for MCF-7 (Breast Cancer) Cells.

Treatment Group	Concentration	Absorbance (570 nm) Replicates	Mean Absorbance	% Cell Viability	% Inhibition
Control	0 µL/mL	2.107	2.114	2.110	2.110
Swarnavari Bhasma	10 µL/mL	1.456	1.452	1.453	1.453
	40 µL/mL	1.112	1.113	1.116	1.113
	100 µL/mL	0.874	0.876	0.871	0.873
5-Fluorouracil (Std.)	10 µg/mL	0.512	0.513	0.515	0.513
	40 µg/mL	0.410	0.412	0.416	0.412
	100 µg/mL	0.306	0.307	0.303	0.305

The detailed raw data and dose-response relationship for each cell line are presented below.

Morphological Analysis of Cell Lines

Morphological examination under a microscope confirmed the cytotoxic effects, showing cell rounding, shrinkage, and detachment from the culture plate surface in treated wells compared to the healthy, confluent monolayer in control wells (Tables 2-5).

DISCUSSION

This study successfully prepared, standardized, and demonstrated the *in vitro* anti-carcinogenic potential of *Swarnavari Bhasma*. The results indicate that the formulation's bioactivity is not due

to a single component but is an emergent property of the complex synergy between its herbo-mineral constituents, realized through traditional Ayurvedic processing.

The analytical characterization provides a strong scientific basis for its potential mechanism. The presence of a significant nanoparticle population (131.8 nm) is crucial, as nano-sized particles are known to enhance cellular uptake, bioavailability, and therapeutic efficacy (Banerjee *et al*., 2016; Chenthamara *et al*., 2019; Hoshyar *et al*., 2016). The unique porous, micro-aggregate structure seen in FESEM images provides a large surface area, which may act as a carrier matrix for the active organic compounds, facilitating their delivery to cancer cells (Bhanja *et al*., 2018; Ezati *et al*., 2025; Shee *et al*., 2024).

The cytotoxic effect is likely multifactorial. The formulation combines several agents with known anticancer mechanisms. The processing with Vincristine directly introduces a potent

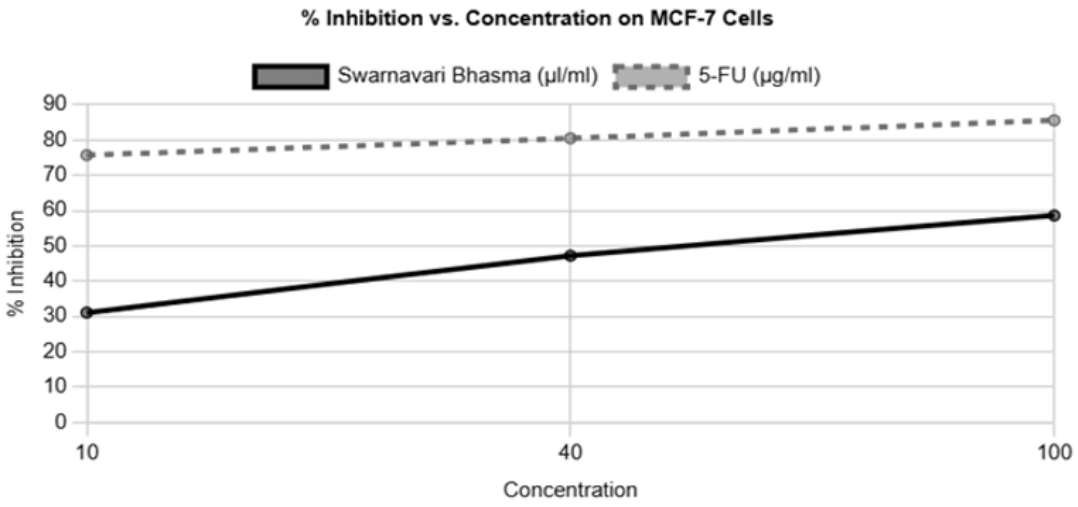

Figure 3: Dose-response curves for MCF-7 cells. The graph shows the percentage of cell viability as a function of the concentration of *Swarnavari Bhasma* and 5-FU.

Table 4: Raw Absorbance and Viability Data for Hep G2 (Liver Cancer) Cells.

Treatment Group	Concentration	Absorbance (570 nm) Replicates	Mean Absorbance	% Cell Viability	% Inhibition
Control	0 µL/mL	2.326	2.325	2.328	2.326
Swarnavari Bhasma	10 µL/mL	1.896	1.895	1.893	1.894
	40 µL/mL	1.456	1.455	1.452	1.454
	100 µL/mL	0.856	0.852	0.851	0.853
5-Fluorouracil (Std.)	10 µg/mL	1.253	1.253	1.254	1.253
	40 µg/mL	0.856	0.851	0.854	0.853
	100 µg/mL	0.421	0.422	0.423	0.422

Table 5: Raw Absorbance and Viability Data for PC-3 (Prostate Cancer) Cells.

Treatment Group	Concentration	Absorbance (570 nm) Replicates	Mean Absorbance	% Cell Viability	% Inhibition
Control	0 µL/mL	2.032	2.033	2.034	2.033
Swarnavari Bhasma	10 µL/mL	1.963	1.962	1.965	1.963
	40 µL/mL	1.752	1.753	1.759	1.754
	100 µL/mL	0.854	0.856	0.851	0.853
5-Fluorouracil (Std.)	10 µg/mL	1.112	1.113	1.114	1.113
	40 µg/mL	0.689	0.681	0.683	0.684
	100 µg/mL	0.512	0.513	0.517	0.514

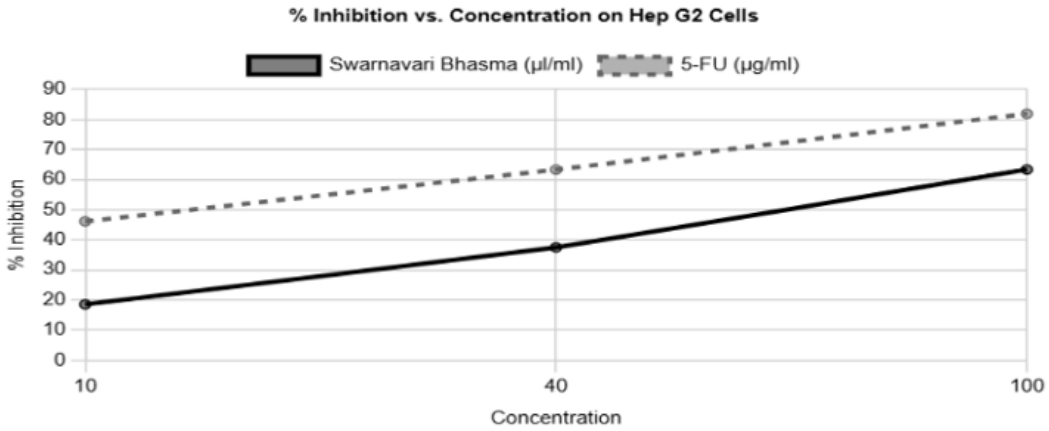

Figure 4: Dose-response curves for Hep G2 cells. The graph shows the percentage of cell viability as a function of the concentration of *Swarnavari Bhasma* and 5-FU.

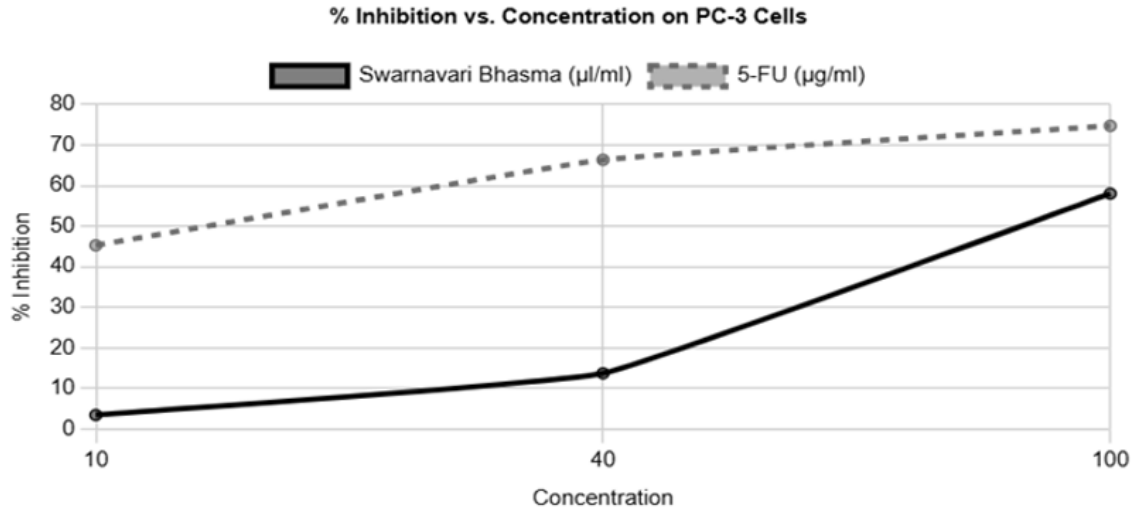

Figure 5: Dose-response curves for PC-3 cells. The graph shows the percentage of cell viability as a function of the concentration of *Swarnavari Bhasma* and 5-FU.

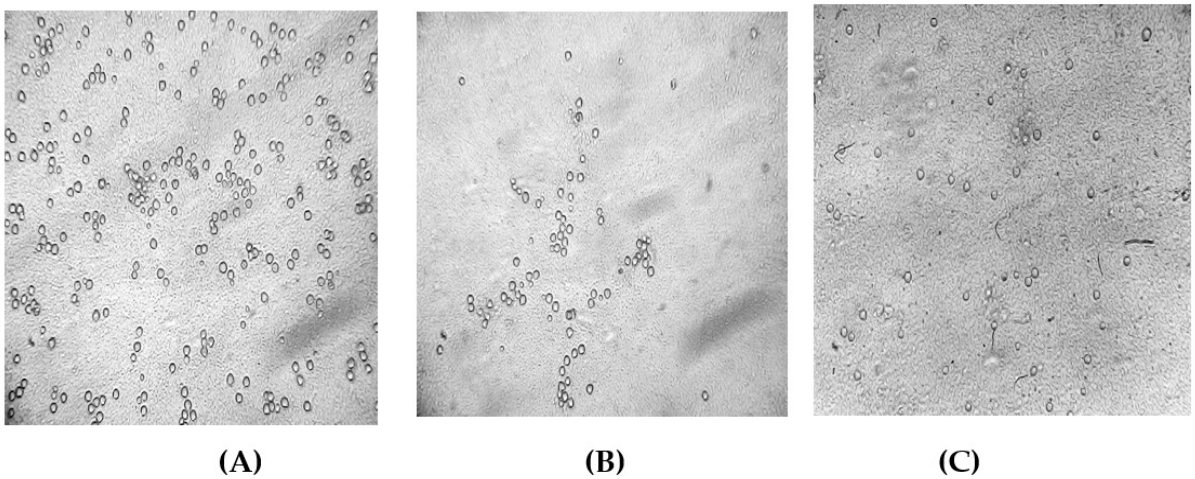

Figure 6: Morphological changes in A549 (Lung Cancer) cells. (A) Untreated control cells showing a healthy, confluent monolayer. (B) Cells treated with 5-FU showing significant cell death and detachment. (C) Cells treated with *Swarnavari Bhasma* (100 µL/mL) showing reduced cell density, rounding, and shrinkage.

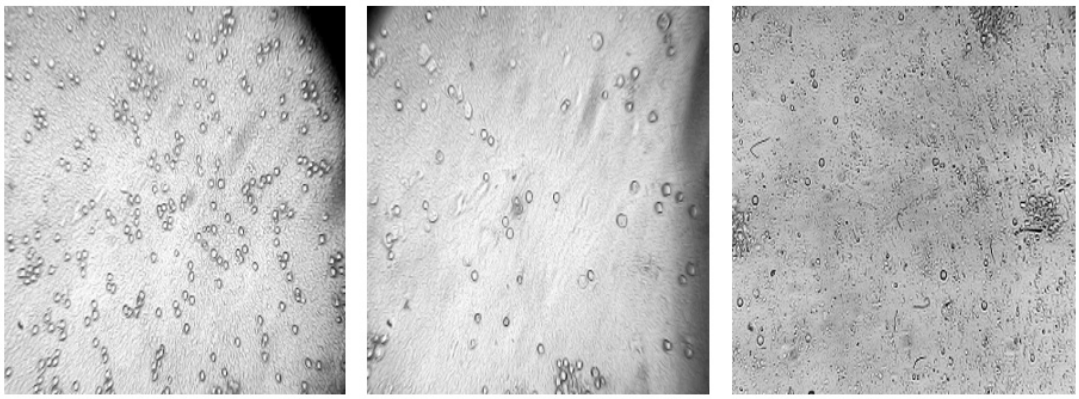

Figure 7: Morphological changes in MCF-7 (Breast Cancer) cells. (A) Untreated control cells. (B) Cells treated with 5-FU. (C) Cells treated with *Swarnavari Bhasma* (100 µL/mL), showing pronounced cytotoxicity.

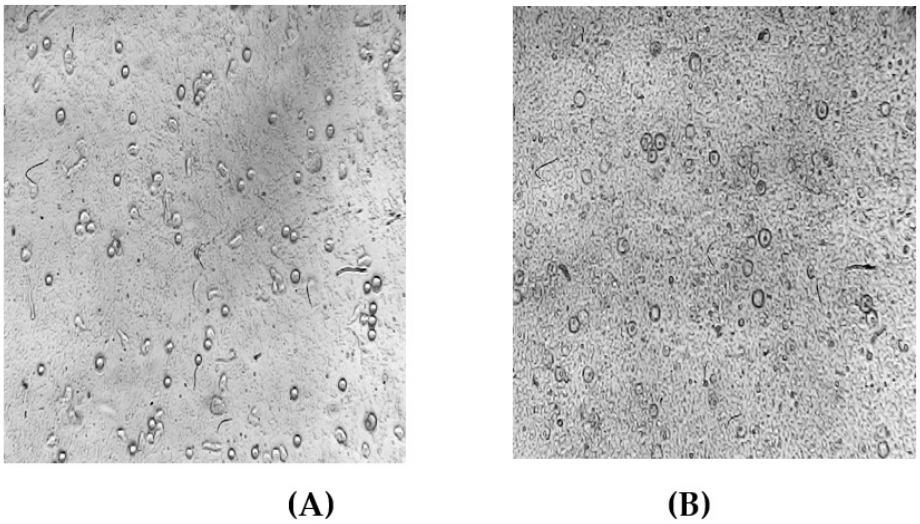

Figure 8: Morphological changes in Hep G2 (Liver Cancer) cells. (A) Untreated control cells. (B) Cells treated with *Swarnavari Bhasma* (100 µL/mL).

mitotic inhibitor that arrests cancer cells in the M-phase of the cell cycle, leading to apoptosis (Dhyani *et al*., 2022; Kothari *et al*., 2016). Curcumin from *Haridra* is known to induce apoptosis and suppress key pro-cancer signaling pathways like NF-κB and STAT3 (Sultana *et al*., 2021; Wang *et al*., 2019; Wilken *et al*., 2011). *Triphala* contributes potent antioxidants like gallic acid, which protect against oxidative DNA damage and can selectively induce apoptosis in cancer cells (Charucharana *et al*., 2025; Patra *et al*., 2020; Prasad and Srivastava, 2020; Sripunya *et al*., 2024). Piperine from *Pippali* increases the bioavailability of other active compounds, particularly curcumin, thereby potentiating the overall effect of the formulation.The high alkalinity of *Kapardika Bhasma* (pH 12.1) suggests a potential to neutralize the acidic tumor microenvironment, a condition known to promote tumor invasion and chemoresistance (Pratti *et al*. 2024).

The differential sensitivity observed across the cell lines (MCF-7>Hep G2>A549>PC-3) suggests that the formulation's efficacy may be dependent on the specific molecular pathways active in different cancer types. The highest sensitivity in the ER-positive MCF-7 cell line is a particularly noteworthy finding that warrants further investigation.

While *Swarnavari Bhasma* was less potent than the pure cytotoxic agent 5-FU, this is expected for a multi-component formulation designed for safety and synergy. Its significant intrinsic activity suggests its potential not as a replacement for chemotherapy, but as an adjuvant or integrative therapy. Its multi-targeted mechanism could help overcome drug resistance, enhance the efficacy of conventional drugs, or allow for reduced dosages, thereby mitigating dose-limiting toxicities.

The primary limitation of this study is it's *in vitro* nature, which cannot account for pharmacokinetics, metabolism, or interaction with the tumor microenvironment and immune system in a living organism.

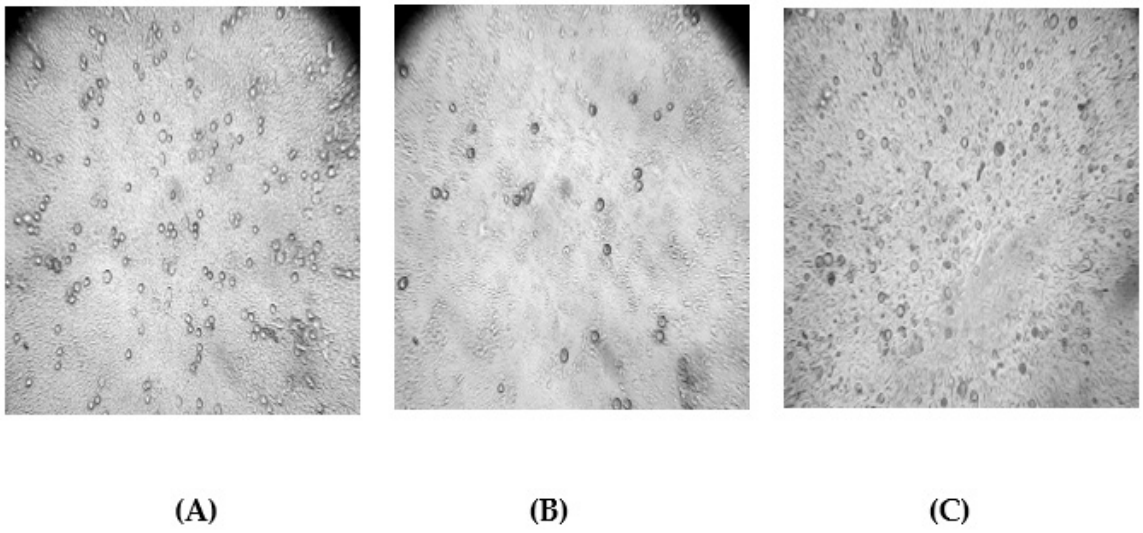

Figure 9: Morphological changes in PC-3 (Prostate Cancer) cells. (A) Untreated control cells. (B) Cells treated with 5-FU. (C) Cells treated with *Swarnavari Bhasma* (100 µL/mL).

CONCLUSION

This research provides a robust scientific validation for *Swarnavari Bhasma* as a potential anti-carcinogenic agent. The study successfully demonstrated that this rationally designed, standardized Ayurvedic herbo-mineral formulation exhibits significant, dose-dependent cytotoxic activity *in vitro* against human lung, breast, liver, and prostate cancer cells. The integration of classical pharmaceutical methods with modern analytical techniques confirmed its unique nano-micro particulate structure and complex chemical composition, providing a basis for its multi-targeted mechanism of action. These promising findings establish a strong proof-of-concept and justify further preclinical investigation, including *in vivo* animal studies, to evaluate its efficacy, safety, and potential as an integrative therapy in oncology.

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ABBREVIATIONS

SM: Swarna Makshika; **FESEM:** Field Emission Scanning Emission Microscopy; **XRD:** X- ray Diffraction; **FTIR:** Fourier Transform Infrared Spectroscopy; **MTT:** 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **5-FU:** 5-Fluorouracil; **IC_{50}:** Half- maximal inhibitory concentration; **LOD:** Loss on Drying; **DMSO:** Dimethyl Sulfoxide; **NCCS:** National Center for Cell Sciences; **FBS:** Fetal Bovine Serum.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL STATEMENT

The study was approved by the Institutional Ethics Committee of Mahatma Gandhi Ayurvedic Medical College, Hospital and Research Centre for *in vitro* Evaluation of anti-carcinogenic activity of *Swarnavari bhasma* (A herbo-mineral formulation) in selected cancer cell lines (Ref. No. MGACCHRC/IEC/SEP-203/743). Cell lines were procured from the National Centre for Cell Sciences (NCCS), Pune, and handled in certified biosafety laboratories according to standard aseptic and disposal protocols.

AUTHOR CONTRIBUTIONS

Dr. Keyur B. Dudhat designed the study, prepared the formulation, performed analysis, and wrote the manuscript. Dr. Anita Wanjari supervised the work and reviewed the manuscript. Dr. Mujahid Khan assisted in methodology and manuscript review.

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