Anticancer activity of *Yashada Bhasma* (bioactive nanoparticles of zinc): A human pancreatic cancer cell line study

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Abstract

Background: Pancreatic ductal adenocarcinoma is the fourth leading cause of cancer-related mortality. Efforts to improve cancer treatment through nanotechnology are at the developmental stage, and it will be gracious if the drug with anticancer property itself is a nanoparticle. *Bhasma* is organomineral complexes which are bioactive nanoparticles. *Yashada Bhasma* (incinerated processed zinc) is widely used in Ayurveda for various diseases, and there are evidence that ZnO nanoparticles are promising antitumor agent. However, no studies have been conducted on the effectiveness of *Yashada Bhasma* in pancreatic cancer. **Materials and Methods:** Two types of test drugs, *Parada Marita Yashada Bhasma* (PMY) and *Vanaspati Jarita Marita Yashada Bhasma* (JMY), were prepared as per the guidelines of pharmaceutics of Ayurveda. Particle size analyses of *Yashada Bhasma* and Zeta potential study was carried out initially. Further human pancreatic cancer cell line (MIA PaCa-2) study was done using *in vitro* sulforhodamine B assay, keeping adriamycinas control. After 48 h of incubation, antiproliferative effects were assessed. **Results:** JMY and adriamycin showed dose-dependent growth inhibition of cancer cells. Both *Yashada Bhasma* samples showed a cytostatic effect at this concentration. **Conclusion:** The study leads to new avenues for cancer treatment by developing such unique and highly effective bioactive nano-sized therapeutic agent.

Keywords: Anticancer, human pancreatic cell line, *in vitro*, nanoparticle, *Yashada Bhasma*, zinc

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is an exocrine pancreatic cancer, which accounts for over 90% of pancreatic cancers and the fourth leading cause of cancer-related mortality. The prognosis is extremely poor, with a 5-year survival rate of around 5%, as the disease is intrinsically very aggressive and highly resistant to the conventional treatment modalities such as radiation and chemotherapy. Despite increasing knowledge in tumor biology, diagnostic tools, and different treatment options, the effectiveness of pancreatic cancer control measures has not improved significantly over the past decade.[1]

Efforts to improve cancer treatment through nanotechnology are at the developmental stage, and it will be gracious if the drug with anticancer property itself is a nanoparticle. *Bhasma* is organomineral complexes, having improved stability, bioavailability, biocompatibility, targeted delivery, and therapeutic efficacy.[2] They are bioactive nanoparticles.

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Yashada Bhasma (incinerated processed zinc) is a widely used medicine in various diseases such as diabetes, Parkinson's disease, and respiratory disorders in Ayurvedic therapeutics,[3] and there are evidence that ZnO nanoparticles are promising antitumor agent in *in vitro* and *in vivo* studies.[4] However, no studies have been conducted on the effectiveness of *Yashada Bhasma* in pancreatic cancer.

Human cancer-derived cell lines are fundamental models used in laboratories to study the biology of cancer and to test the therapeutic efficacy of anticancer agents.[5] Anticancer activity of two different types of *Yashada Bhasma* was analyzed using human pancreatic cancer cell lines.

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Materials and Methods

Preparation of *Yashada Bhasma* (test drugs)

Test drugs, *Parada Marita Yashada Bhasma* (PMY) and *Vanaspati Jarita Marita Yashada Bhasma* (JMY), were prepared as per the classical guidelines in Ayurveda.[6] Samples differ in their method of preparation. PMY sample was prepared after making *Dhatu Pishti* (paste of mineral) with *Parada* (mercury/Hg). The current *Dhatu Pishti* was prepared by mixing purified mercury[7] and purified zinc[8,9] in its molten stage and grinding in the presence of herbal liquid media until amalgamation. JMY sample was prepared after doing *Jarana* (roasting until it gets converted to fine powder) in *Apamarga Panchanga* (*Achyranthes aspera* Linn. – coarse powder of whole plant).[10] After this procedure, both samples were subjected to incineration in electric muffle furnace to prepare *Bhasma*.[11]

Particle size analysis of test drugs

Particle size analysis of *Yashada Bhasma* was done by dynamic light scattering method (DLS). This was followed by zeta potential study, to assess the potency of these particles.[12]

Cell lines and cell culture

Human pancreatic cancer cell line MIA PaCa-2[13] was developed in the Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Mumbai and the study was carried out in the same center. It was used as *in vitro* sulforhodamine-B (BSRB) assay models to study carcinogenesis in pancreatic ductal adeno carcinoma (PDAC). The cell lines were grown in Roswell Park Memorial Institute 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. Cells were inoculated into 96-well microtiter plates in 100 µL at plating densities. After cell inoculation, the microtiter plates were incubated at 37°C, for 24 hrs prior to addition of experimental drugs.

Sample preparation

Test drugs were dissolved in dimethyl sulfoxide at 100 mg/ml and diluted to 100 µg/ml, 200 µg/ml, 400 µg/ml, and 800 µg/ml. Later, it was tested at four different concentrations, i.e. 10 µg/ml, 20 µg/ml, 40 µg/ml and 80 µg/ml in two different cell lines. Antiproliferative effects of test drugs were analyzed, keeping adriamycin/doxorubicin as a standard control.

Estimation of cell growth assay

As and when the drugs were added to the cell lines, plates were incubated at standard conditions for 48 h and assay was terminated by the addition of cold trichloroacetic acid. Percentage growth was calculated on a plate-by-plate basis for test wells relative to control wells using six absorbance measurements read at wavelength 540 nm with 690 nm reference. Percentage growth was calculated as the ratio of average absorbance of the test well to the average absorbance of the control wells, i.e., ([Ti/C] ×100%) at each of the drug concentration levels. The concentration of drug causing 50% cell kill (LC 50), 50% inhibition of cell growth (TG 50) and total inhibition of cell growth (TG) were analyzed.[14]

Results

Particle sizes of both samples of test drugs were reduced significantly. JMY particles were distributed within the range of ≤1 nm to 2300 nm. Z averages (average particle size) of JMY samples were 2300 (d.nm) [Figure 1] and maximum number of particles has zeta potential within the range −22.4 ± 7.50 with −21.1 as average zeta potential [Figure 2]. PMY particles were distributed within the range of ≤1 nm –10,000 nm. Z average was 2647 nm [Figure 3] and maximum number of particles have zeta potential within −14.4 ± 7.50 range with −9.20 as average zeta potential [Figure 4].

The PMY sample inoculated cell line exhibited an average cell growth of 103.7%, 104.5%, 103.7% and 101.7% at a concentration of 10 µg/ml, 20 µg/ml, 40 µg/ml and 80 µg/ml, respectively, while JMY sample inoculated cell line exhibited 105%, 103.2%, 104.2% and 2.8% average cell growth at a concentration of 10 µg/ml, 20 µg/ml, 40 µg/ml and 80 µg/ml, respectively. The drug JMY exhibited a 50% growth inhibition at a concentration of 56.7 µg/ml and more than 97% growth inhibition at 80 µg/ml. At the same time, other drug PMY exhibited 50% growth inhibition at a concentration >80 µg/ml only. Adriamycin cell line exhibited a cell growth of − 24.6%, −50.1%, −65.5% and − 54.6% at a concentration of 10 µg/ml, 20 µg/ml, 40 µg/ml and 80 µg/ml, respectively and showed cytotoxicity (LC 50) at 41.6 µg/ml. That is, the standard drug adriamycin showed total growth inhibition at a concentration of 10 µg/ml. Both test drugs and adriamycin showed dose-dependent inhibition of cancer cell growth. Test drugs did not exhibit cytotoxicity but showed cytostatic property [Table 1 and Figures 5-9].

Discussion

Zinc plays a wide range of functions in the organism, including DNA synthesis, cell division, and protein synthesis.[15] The administration of zinc oxide nanoparticles exhibited a promising preclinical anticancer efficacy in Hepatocellular carcinoma (HCC).[16] An *in vitro* and *in vivo* study of a novel zinc complex, zinc N-(2 hydroxyacetophenone) glycinate, is proved to overcome multidrug resistance in cancer.[17]

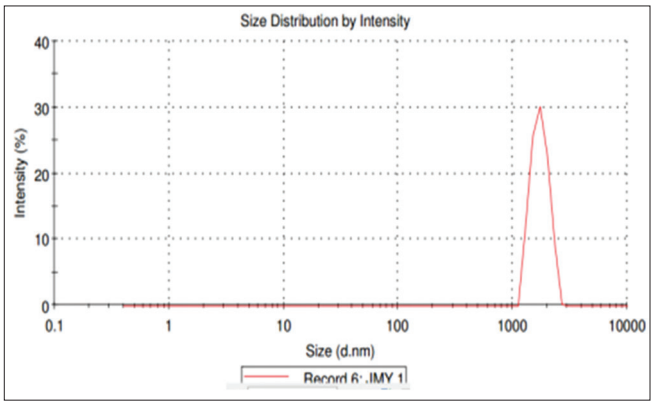

Figure 1: Particle size analysis of *Vanaspati Jarita Marita Yashada Bhasma*

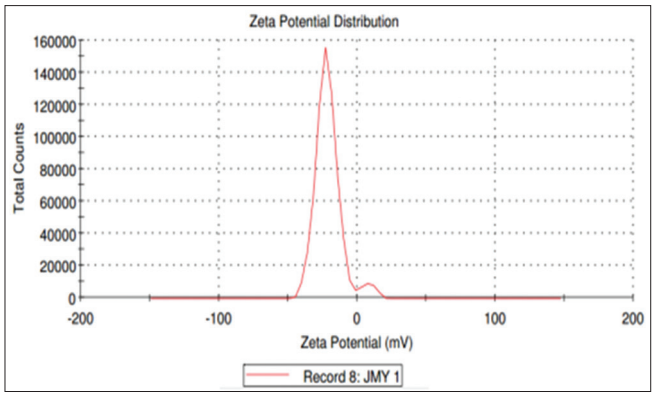

Figure 2: Zeta potential of *Vanaspati Jarita Marita Yashada Bhasma*

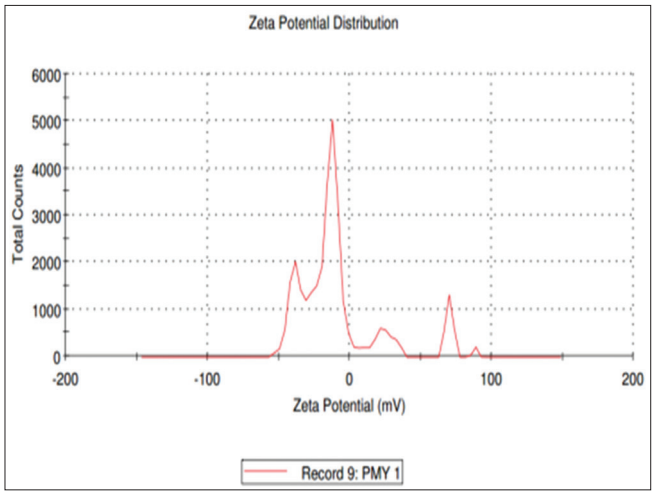

Figure 4: Zeta potential of *Parada Marita Yashada Bhasma*

Adriamycin with zinc supplement ($ZnCl_2$) was proved to be effective in suppressing adriamycin-resistant mammary tumor. The combination was superior to either adriamycin or zinc alone due to the effect of zinc on p53 functionality.[15] Although the previous studies point toward the anticarcinogenic property of zinc nanoparticles, they can elicit many immunological reactions. Screening for this variety of potential adverse conditions can be a challenge, and it is often difficult to predict which assays will be most relevant for a given formulation.[18]

Processing of zinc assures more safety, increased therapeutic efficacy, better bioavailability, and finer particle size. According to Ayurveda, cell division and multiplication are controlled by "*Vata Dosha"* (body humor). Hence, the uncontrolled cell division is a result of imbalance of *Vata Dosha*, which results in neoplasm.[19] The peculiar property of *Yashada Bhasma* to control vitiated *Vata*, i.e. *Ekanga* and *Sarvanga Vata*[20] (localized or generalized) helped in preventing neoplasm of pancreatic cells. Properties of *Yashada Bhasma*[21] are enlisted in Table 2.

The scientific community has increasingly recognized that cell line integrity is critical for maintaining high standards in research and so *in vitro* was preferred.[22] The sample prepared with prior *Jarana* (roasting with herb), i.e., JMY

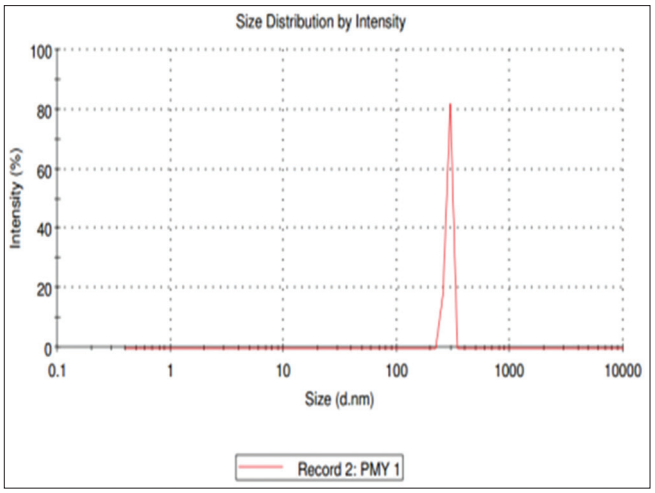

Figure 3: Particle size analysis of *Parada Marita Yashada Bhasma*

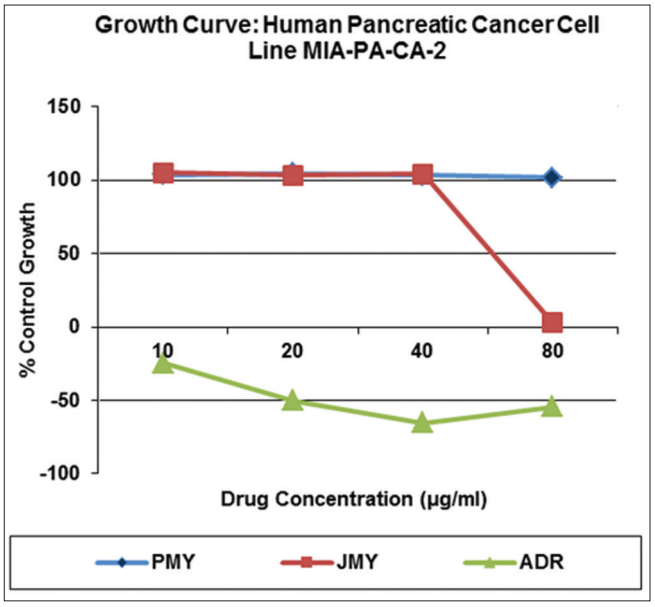

Figure 5: Graphical representation of growth Inhibition. PMY: *Parada Marita Yashada Bhasma* and JMY: *Vanaspati Jarita Marita Yashada Bhasma*, ADR: Adriamycin

sample showed better inhibition of cell growth. Based on the analytical parameters, compound of JMY sample differs from PMY sample and they vary in several factors such as particle size, zeta potential, zinc percent, and level of trace elements. Maximum numbers of particles in JMY were in 1726 ± 297.3 nm range and PMY was in 288 ± 15.53 nm range. Although both the samples are found to be in nanorange, PMY samples showed comparatively lesser particle size.

Surface charge definitely affects cellular uptake, toxicity, and biodistribution of nanoparticles. Inhibitory activity of JMY sample may be attributed to its high negative zeta potential compared to PMY sample. Due to the enhanced permeability and retention effect of nanoparticles, it can accumulate in tumor tissues which have a leaky vasculature and compromised lymphatic drainage.

Table 1: Growth percent of cancer cells at different concentration

Samples	Human pancreatic cancer cell line MIA-PaCa-2 Percentage control growth															
	Experiment 1				Experiment 2				Experiment 3				Average values			
	Drug concentrations (μg/ml)															
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
PMY	100.0	100.5	101.0	99.0	105.0	107.4	104.2	101.0	106.2	105.8	105.1	105.0	103.7	104.5	103.4	101.7
JMY	102.1	99.7	98.9	−43.6	106.2	105.5	104.4	10.9	106.7	104.5	109.6	41.2	105.0	103.2	104.3	2.8
ADR	1.5	−56.4	−69.5	−67.6	−39.3	−53.8	−64.1	−56.7	−35.8	−40.2	−62.7	−39.3	−24.6	−50.1	−65.5	−54.6

PMY: *Parada Marita Yashada Bhasma*, JMY: *Vanaspati Jarita Marita Yashada Bhasma*, ADR: *Adriamycin*

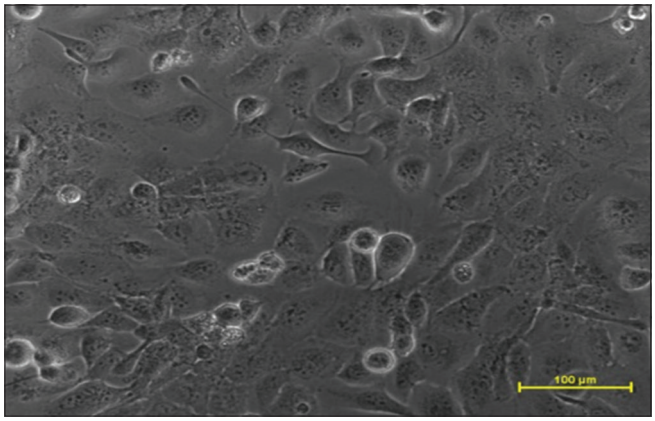

Figure 6: MIA-PA-Ca-2 of control

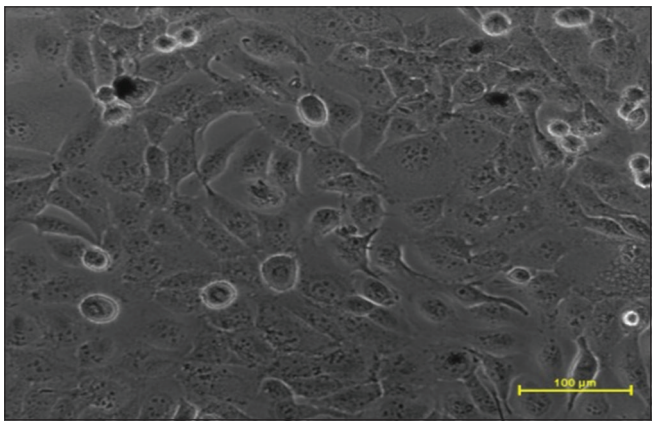

Figure 8: MIA-PA-Ca-2 of *Parada Marita Yashada Bhasma*

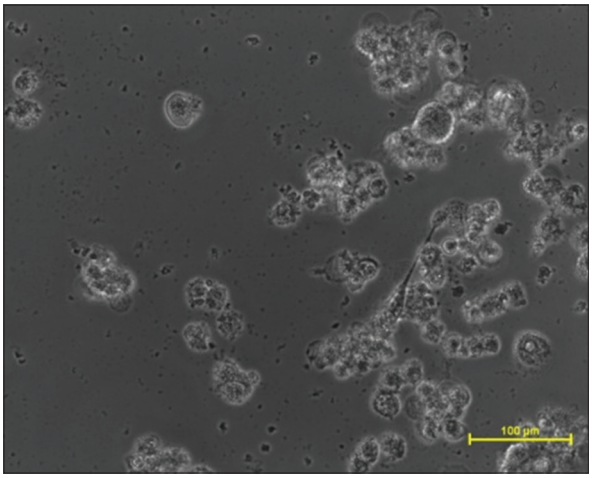

Figure 7: MIA-PA-Ca-2 of *Vanaspati Jarita Marita Yashada Bhasma*

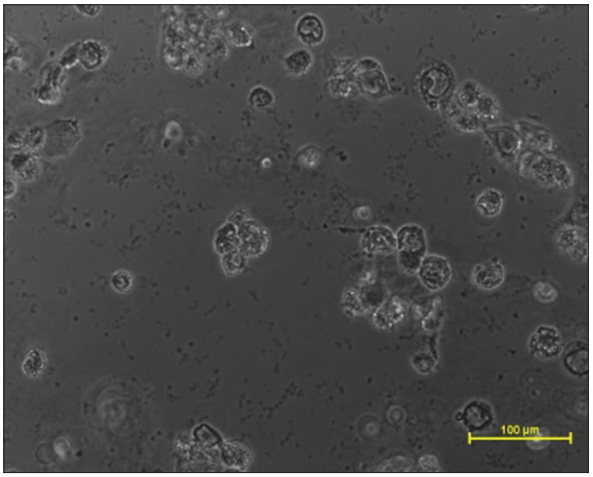

Figure 9: MIA-PA-Ca-2 of positive control

The concentration (ppm) of micronutrients such as Mn, Ni, and Cr present in JMY sample were comparatively higher to that in PMY sample in inductively coupled plasma-atomic emission spectroscopy analysis.[12] Micronutrient synergy inhibits angiogenesis and metastasis.[23]

JMY sample was detected as ZnO and PMY sample as ZnS in XRD findings as shown in Figures 10 and 11.[12] In previous studies too, ZnO nanoparticles have shown antitumor property. The contribution of *Jarana* media cannot be excluded until higher studies are carried out. Here, the media used for *Jarana* process is *Apamarga Panchanga* (whole plant of *Achyranthes aspera* Linn.), a plant rich in *Kshara* (alkaline) property. *Kshara* corrects the cellular level metabolism due to the properties such as *Tikshna* (penetrating), *Ushna* (hot), *Deepana, and Pachana* (improves appetite and digestion).[24] As the test drug has not reported any cytotoxicity, this will result in no damage to the healthy tissue during targeted drug delivery.

As adriamycin and *Yashada Bhasma* have different toxicity profile and dosage, drug efficacy cannot be compared on concentration basis. Since cell line studies cannot be a base

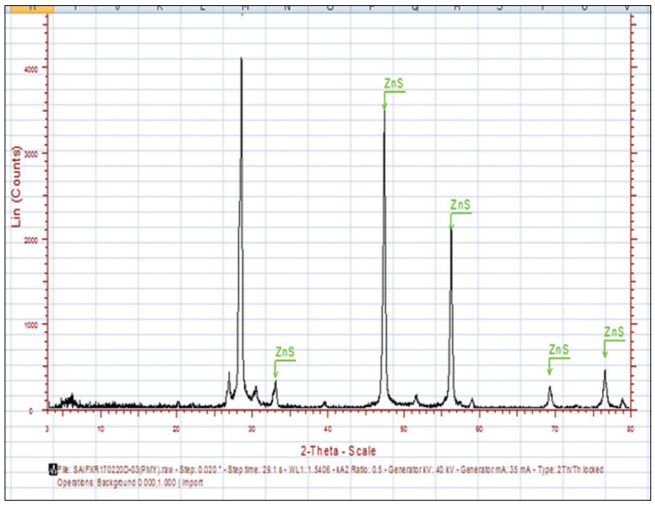

Figure 10: X-ray diffraction image of *Parada Marita Yashada Bhasma* sample

Table 2: Pharmacodynamics of *Yashada Bhasma*

Pharmacodyanamics	Properties of Yashada Bhasma[21]
Rasa (taste)	*Kashaya Katu* (astringent and pungent)
Veerya (potency)	*Sheeta* (cold in potency)
Vipaka (after digestion)	*Katu* (pungent)
Guna (property)	*Balya* (endure strength)
Doshaghnata (pacification of vitiated body humors)	*Vata*, *Pitta*, and *Kapha* (all body humors)
Rogaghnata (pacification of disease)	*Netra vikara* (eye diseases), *Prameha* (diabetes), *Pandu* (anemia), *Kasa Shwasa* (respiratory disorders), *DushtaVrana* (nonhealing wounds), *Shrama* (exertion), *Avasada* (generalized weakness), *Rajasrava* (menorrhagia), *Kampavata* (parkinson's disease), and many disorders with imbalance of *Kapha* and *Pitta*[21]

for human dose, *in vivo* studies are preferred. As per Ayurvedic classics, the dosage of *Yashada Bhasma* is 125–250 mg.[25] Furthermore, in view of safety, expense, and therapeutic spectrum, *Yashada Bhasma* administration is devoid of such predicament. Higher level studies are recommended to detect the effect and combination effect of *Yashada Bhasma* with conventional anticancer drugs.

Conclusion

Yashada Bhasma prepared with prior *Jarana* (JMY) acts as a cytostatic drug in human Pancreatic ductal adenocarcinoma, due to their ability to induce cell growth arrest. This may open up new possibilities for cancer control. The current findings admit new assurance for the treatment of pancreatic cancers by developing a unique and highly effective therapeutic agent.

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Conflicts of interest

There are no conflicts of interest.

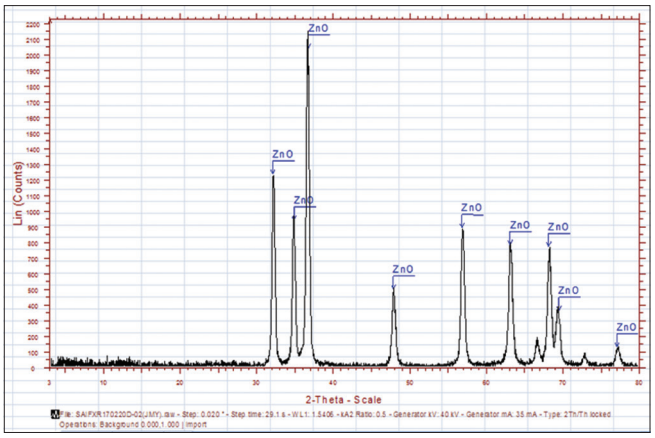

Figure 11: X-ray diffraction image of *Vanaspati Jarita Marita Yashada Bhasm*

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