

BIOMIMETIC SYNTHESIS, CHARACTERIZATION AND ANTICANCER ACTIVITIES OF ZIRCONIUM NANOPARTICLES USING VINCA ROSEA AQUEOUS EXTRACT

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Abstract: The importance of vinca rosea leaves and flower with different metals as revealed by various literature resources, we planned to carry out synthesis of zirconium nanoparticles using the above extract. Zirconium nanoparticle formations were characterized by UV, FTIR, TEM, TG and XRD. The UV spectra results show a strong resonance centre and surface of zirconium nanoparticles at 299nm. XRD and TEM studies revealed that the synthesized nanoparticles are spherical in shape with average particles size around 0.27nm to 100nm. However, there is limited information about antitumor potential. Therefore, this study focused on determining cytotoxic effects of ZrNPs on MDA-MB-231 breast cancer cells and its mechanism of cell death. The zirconium nanoparticles using an aqueous extract of vinca leaves show potential anticancer activity on MDA-MB-231 breast cancer cells. The toxicity was evaluated using cell viability, metabolic activity, and oxidative stress. MDA-MB-231 breast cancer cells were treated with various concentrations of ZrNPs (5 to 25 $\mu\text{g}/\text{mL}$) for 24 h. We found that ZrNPs inhibited the growth in a dose-dependent manner using MTT assay. ZrNPs showed dose-dependent cytotoxicity against MDA-MB-231 cells through activation of the lactate dehydrogenase (LDH), caspase-3, reactive oxygen species (ROS) generation, eventually leading to induction of apoptosis which was further confirmed through resulting nuclear fragmentation. The present results showed that ZrNPs might be a potential alternative agent for human breast cancer therapy.

Key words: Vinca Rosea, Anticancer, Zirconium nanoparticles, XRD etc.

Introduction

In the present scenario, nanotechnology is an important enabling active area of research in modern material science. Nanoscience has emerged in the past few years as an interdisciplinary science [1], in the fields of targeted drug delivery and cancer [2,3]. Nanoparticles show completely new or improved properties and have the wide scope for their diversified applications based on specific characteristics such as size, distribution, and morphology. Historically, the synthesis of metallic nanoparticles utilized chemical reducing agents such as hydrazine, sodium citrate, and sodium borohydride to create uniform suspensions. However, the chemical method is harmful, expensive and time consuming and can be dangerous to human

health and the environment because of the application of hazardous substances [4]. Therefore, there is a growing need to develop cost-effective and environment friendly approaches for rapid synthesis of nanoparticles.

Zirconia nanoparticles are of great interest due to their improved optical and electronic properties with application as a piezoelectric, electro-optic and dielectric material [5]. Zirconia is also emerging as an important class of catalyst [6]. The study aimed at incorporating the beneficial effects of both herbal and inorganic metal ion for therapeutic efficiency. *Vinca rosea* with its well known traditional properties was herb of choice to evaluate its anticancer activity. Though the anticancer properties of *Vinca rosea* leaves have been proven but its efficacy in the form of ZrNPs has not been studied yet. This is the first study to report the anticancer activity of green synthesized zirconium nanoparticles prepared using *Vinca rosea* leaves extract.

Material and Methods

Vinca rosea leaves (25 gms) was collected from the open field at Vijayapur and washed with double distilled deionizer water and dried in shadow. The skin was removed from the tomato and whole was squeezed to get the juice. This was dissolved in distilled water and filtered using wattman filter paper. zirconium nitrate (0.1M) was used as precursor for the synthesis of thorium nanoparticles.

Characterization of Zirconium nanoparticles

UV-Vis Spectra analysis: Ultraviolet visible spectrophotometer (UV-Vis) refers to absorption spectroscopy in the UV-Visible spectral region. This means it uses light in the visible and adjacent (near UV and near- infrared (NIR) ranges. The absorption in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. Ultraviolet visible spectrophotometer (UV-Vis) is procured from Shimadzu. A small aliquot of the sample was taken for UV-Vis spectrum analysis (200-800nm).

TEM Analysis of Zirconium Nanoparticles: Transmission electron microscopy (TEM) is a microscopy technique where by a beam of electrons s transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen and the image is magnified and focused onto an imaging device. Transmission electron microscopy measurements were performed on Jeol/JEM 2100 model 1200EX instrument operated at an accelerating voltage at 200 kV.

Fourier Transform Infrared: Dried powder of the ZrNPs was subjected to analyze the presence of possible functional groups for resulting in formation of ZrNPs w using Fourier transform infrared (ATR schimadzu Japan) spectroscopy.

X-Ray Diffraction Analysis: To determine the nature and size of the synthesized ZrNPs w, X-ray diffraction (XRD) was performed using on an Xpert Pro MPD, which was operated at a voltage of 40 kV and current of 40mA with Cu-Ka radiation.

TGA / DTA of Zr NPS: Thermo gravimetric (TGA) and Differential thermal analysis was carried out using a Perkin Elmer Thermal analyzer (TG/DTA diamond) in presence of N₂ with 10°C/min heating rate from room temperature to 800⁰C to optimize the heat treatment programme.

Anticancer Activity: Human Breast Cancer Cell Line [MDA-MB-231]

The MDA-MB-231 human breast cancer cell line was carried out in KLE basic science research centre in Belagavi. They were cultured in Dulbecco's modified eagle's medium(DMEM; Gibco) supplemented with 10% fetal bovine serum(FBS;Gibco), penicillin and streptomycin (both from Sigma) in a 50% carbon dioxide (CO₂) cell incubator at 37⁰C to reach 70-80 % confluence[10].Human white blood cells (WBCs) were extracted from a blood sample of a healthy donor by RBC lysis buffer. WBCs were then washed with FBS-free DMEM and adjusted to 1X10⁴ cells/well for analysis [7, 8].

Results and Discussions

UV-Vis Spectra analysis: Fig.1 shows the absorption spectra of ZrNPs. The absorption spectrum showed the absorption peak at 299nm corresponding to the characteristic band of zirconium nanoparticles. In general, the emissions that appear at short wavelength can be ascribed to the excitation from the near band edge transitions [9].



Fig.1

HRTEM Analysis of Zirconium Nanoparticles and SAED Pattern:

TEM images of ZrNPs (1-20nm) show change in the overall particle morphology and formation of denser zirconia particles(Fig.).The discrete, individual particles observed particles observed in the as-prepared ZrNPs have given way to more aggregated structure in which the individual particles can barely be discerned. SAED analysis of the ZrNPs shows sharp ring patterns that could be indexed based on the monoclinic structure [10].

Fig.2

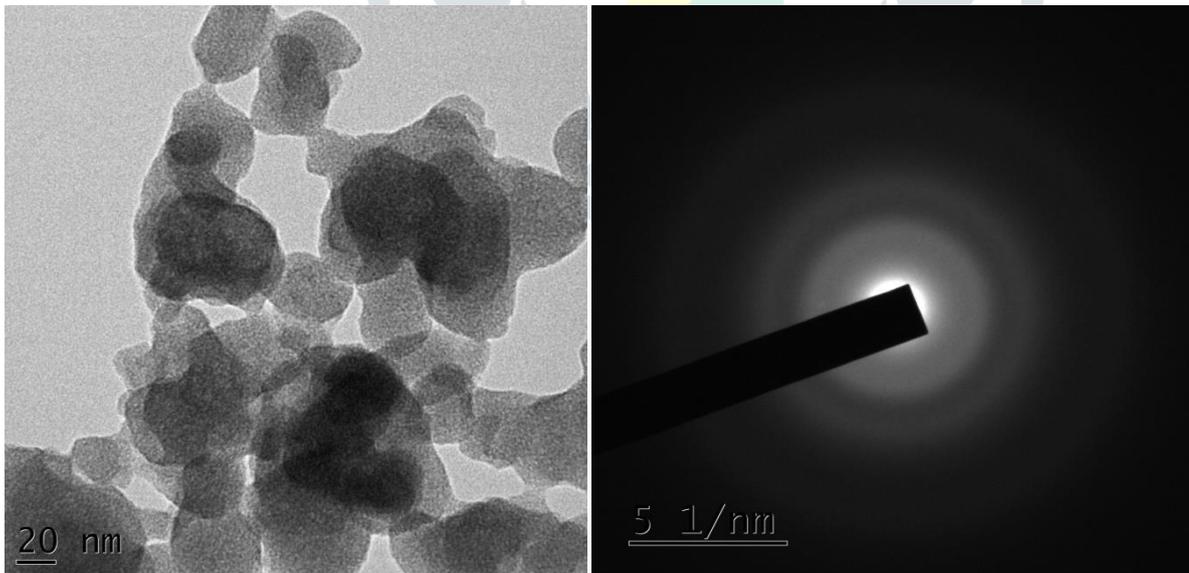
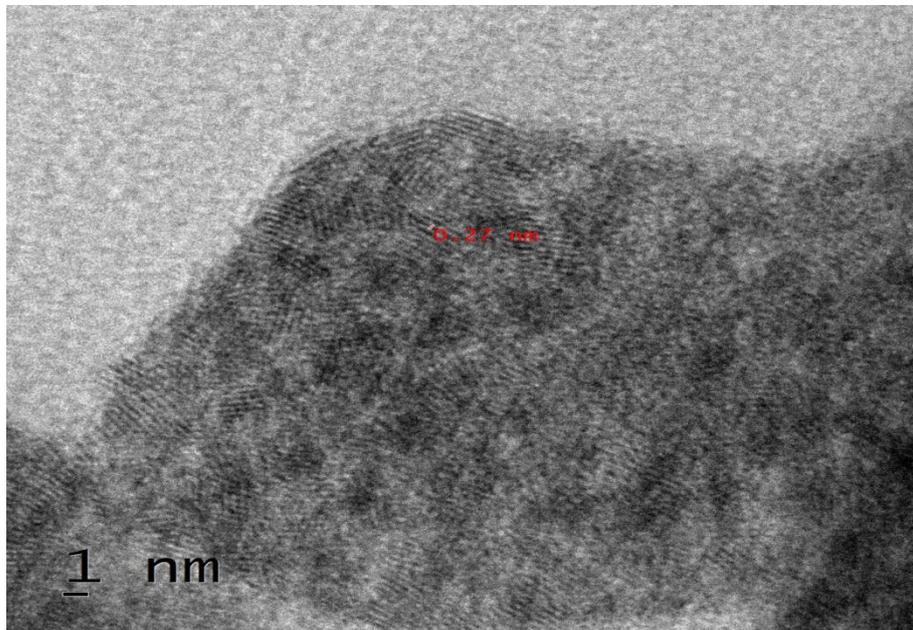


Fig.3

Fig.4

Fourier Transform Infrared: FTIR analysis was performed to find functional groups and for capping and stabilization of the synthesized ZrNPs. In Fig.5,the FTIR spectrum of vinca

rosea aqueous extract showed the absorption band at 3410 cm^{-1} (C-H stretch), 1600 cm^{-1} (N=O stretch), 1390 and 1310 cm^{-1} (N=O bend), 1110 cm^{-1} (C-O bond) and N=O bend at 690 cm^{-1} vibrations are common in vinca rosea extract indicating that these biomolecules were involved in reduction and capping of zirconium nanoparticles [11].

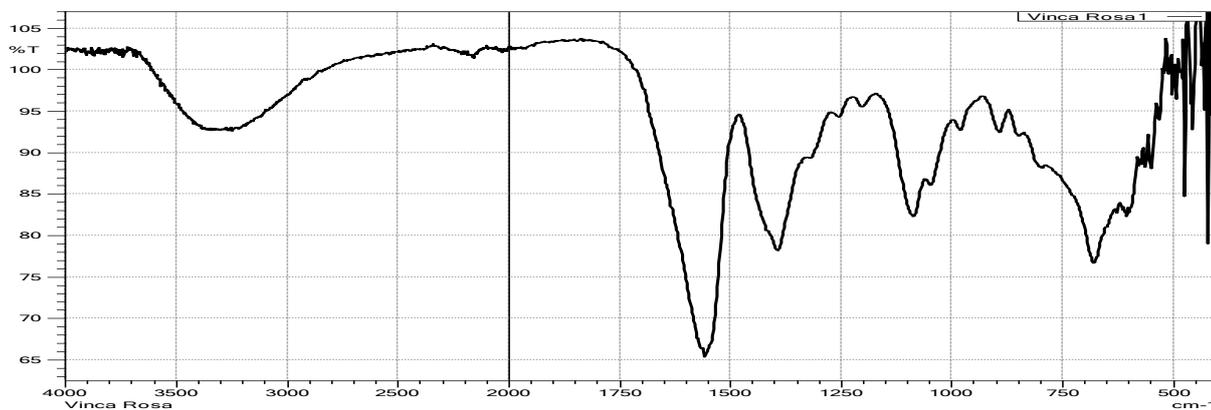


Fig.5

X-Ray Diffraction Analysis: XRD pattern of ZrNPs is given in Fig.6. XRD has been used to characterize the structure of Zirconium nanoparticles. The various peaks in the XRD pattern could be assigned the face centered cubic structure. The XRD pattern showed different intensity peaks in the whole spectrum of 2θ values from 13.013° to 71.48° for the Vinca rosea. The Vinca rosea nanoparticles were indexed as (111), (200), (220), (311), (222) and (400). The average crystalline size was determined using Scherrer's equation [12]. The average crystalline size of ZrNPs was 15.73 nm.

P1

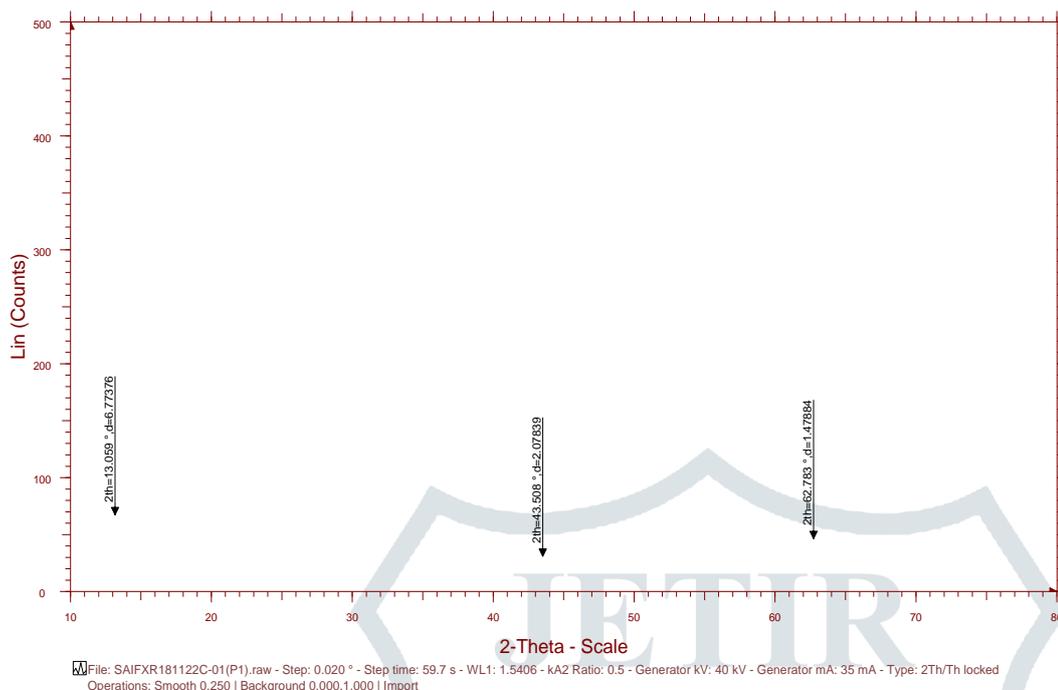


Fig.6

TGA and DTA of ZrNPS: Figure.7. Shows recorded thermogram of zirconium nanoparticles in air up to 856⁰C. Thermogram shows loss of water in first step corresponds to 17.89% with observed weight loss matching with the calculated one. The second weight loss between 250 to 470⁰C, is due to the decomposition of organic residues. Starting at the temperature of 480⁰C, the remaining material was almost pure ZrO₂ nanoparticles with some left-out carbonaceous product formed as a result of overheated product. The sharp and strong peaks in DTG curve with maxima at 94.26 and 391.32⁰C . This could be attributed to the oxidation and the decomposition of organic compounds in the sample [13].

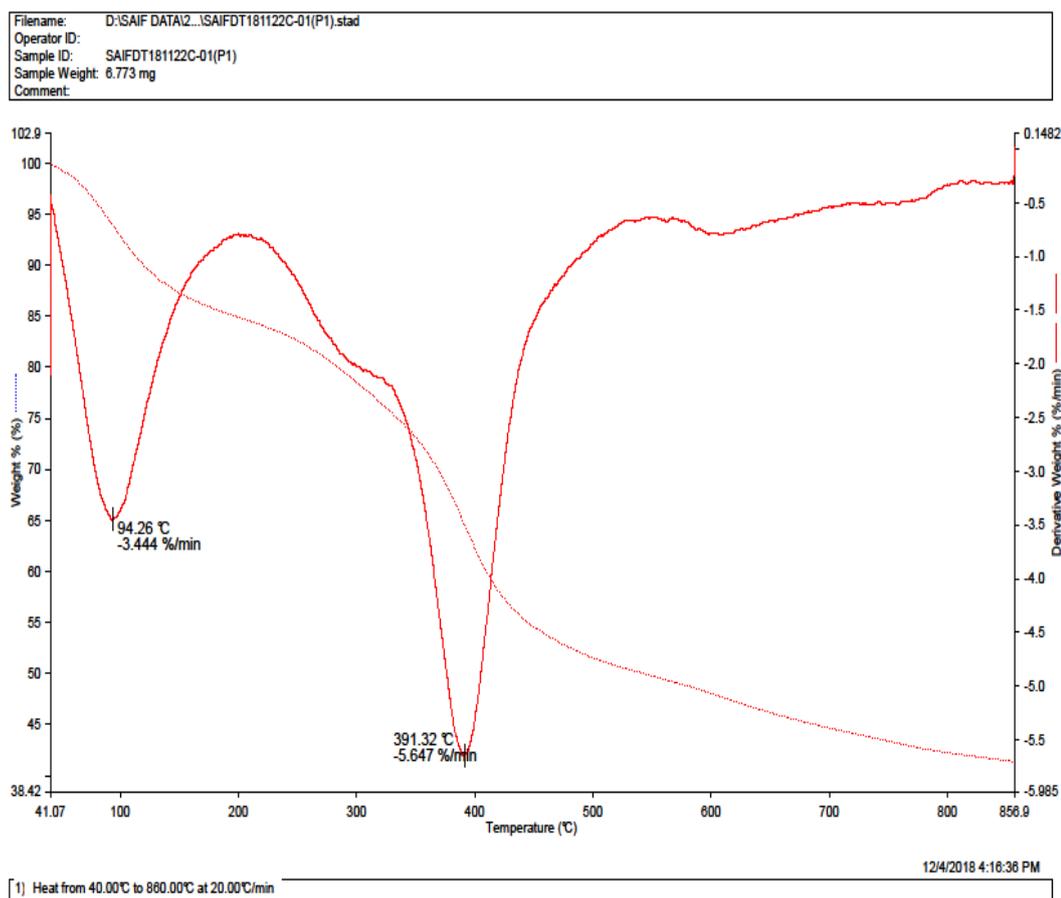


Fig.7

Cell Line- Human Breast Cancer Cell Line [MDA-MB-231]

Table.No.1. Anticancer activity cabbage nanoparticles with ZrNPs was carried out against human breast cancer cell line [MDA- MB-231] at different concentrations to determine the Cell viability (%) by MTT assay. Results of different concentrations (62.5, 125, 250, 500 and 1000 $\mu\text{g/ml}$) of cabbage nanoparticles with Zirconium are tabulated in Table 1. MTT assay of cabbage nanoparticles with ZrNPs demonstrated significant growth inhibition in human breast cancer cell line [MDA-MB-231]. The percentage of cell viability was found to be 61.45, 56.87, 52.1, 47.91 and 45 respectively at concentrations of 62.5, 125, 250, 500 and 1000 $\mu\text{g/ml}$ of test compound.

Table.1

Compound	Concentration ($\mu\text{g/ml}$)	Cell Viability (%)
Human Breast Cancer Cell Line	1000	45
	500	47.91
	250	52.1
	125	56.87

MDA-MB-231	62.5	68.45
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