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Biosynthesis, Characterization and Anti-Cancer Activities of Thorium Nanoparticles Using Ananas Comosus (Pineapple) Extract

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Abstract: *Chemicals typically available in plants have the capability to reduce thorium and to create thorium nanoparticles. We report the preparation of thorium nanoparticles with sizes between 1µm to 100 nm from thorium nitrate using fruit extract collected from pineapples as reducing agents. The resulting nanoparticles were characterized by Scanning Electron Microscopy, Fourier Transform Infrared (FTIR), EDAX, Powder X-ray Diffractions (PXRD) and Anti-cancer activity.*

Key Words: Nanoparticles, Pineapple, Biosynthesis, PXRD, FTIR, EDAX, Anticancer activity

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I. Introduction:

Among the various nanoparticles, metal nanoparticles assume special importance because they are easier and cheaper to synthesize and are most promising in application. The need for biosynthesis of nanoparticles rises because the physical and chemical processes are costly, hazardous, longer time, tedious to isolate nanoparticles. These green synthesis methods have several advantages over other methods namely cost effectiveness, simplicity, use of less temperature, the usage of less toxic materials, moreover it is compatible for medical and food applications. Therefore, in the search for cheaper pathways for nanoparticle synthesis, scientists used microorganisms and then plant extracts for synthesis. Nanotechnology is mainly concerned with synthesis of nanoparticles of variable sizes, shapes, chemical compositions and controlled dispersity with their potential use for human benefits [1].

The increased surfaces of nanoparticles are responsible for their different chemical, optical, mechanical, magnetic properties as compared to bulk materials [2]. Physical and chemical methods of synthesis of nanoparticles are expensive, time consuming, labour intensive and also require more energy. These methods are potentially hazardous to the environment and living organisms due to use of toxic reducing and stabling agents [3]. Development of green nanotechnology is creating interest of researchers towards eco-friendly biosynthesis of nanoparticles. Biomolecules present in plant extracts can be used to reduce metal ions into nanoparticles in a single step green synthesis process. This biogenic reduction of metal ion is quite rapid, readily conducted at room temperature and pressure and easily scaled up [4].

Actinide based nanoparticles and particularly actinide oxides have recently been proposed as potential "precursors "to synthesize innovative nonstructured nuclear fuels at low temperature [5]. In this paper, we report on the synthesis of thorium nanoparticle with pineapple extract and were characterized by using SEM, FTIR, EDAX and PXRD. This study demonstrated the Th Nps from pineapple could be the potential to further develop a chemosnsitizer in the treatment of cancers.

II. Materials and Methods

Synthesis of Thorium Nanoparticle

Pineapple fruits were purchased from the Vijayapur market. The extraction sample was prepared by extracting the juice of the pulpy fruit, sieving it and storing it for the synthesis of ThNPs. Both fresh and refrigerated juices were used and they yielded similar results. An aqueous solution of 0.1M thorium nitrate was prepared. Pineapple juice was added to thorium nitrate at volumetric ratio of 1mL pineapple extract to 10mL thorium nitrate. A colour change was observed within approximately 5 minutes of the reaction.

SEM Analysis of Thorium Nanoparticles: Scanning Electron Microscope (SEM) analysis was done using (JEOL Model JSM - 6390LV) SEM machine. The films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid.

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

EDAX Analysis: EDAX (Energy Dispersive X-ray) analysis of purified ThNPs was carried out using the instrument for confirming the elemental composition of the sample.

X-Ray Diffraction Analysis: To determine the nature and size of the synthesized ThNPs, 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.

Anticancer Activity: A 375 cells were sub cultured in DMEM (Gibco) by adding 10% fetal bovine serum (FBS), pencillin (100 IU/ 100ml), and streptomycin (100 µg/ml) and incubated at 37 ^{0}C in a CO2 incubator (5%). Stock suspension of TNPs (1mg/ml) in DMEM (added with 10% FBS) was diluted to concentrations (5-40 µg/ml) for morphology of cells, cytotoxicity, comet tests. For each experiment, the suspension of Th NPs was freshly prepared, diluted to suitable doses and instantly exposed to the cells. Culture medium without Th NPs served as the control in each experiment.

III. Results and Discussion

Characterization of biosynthesized thorium nanoparticles

SEM Analysis

Scanning electron microscopy provided further insight into the morphology and size details of the thorium nanoparticles. Experimental results showed that the diameter of biosynthesized thorium nanoparticles were about 1-100nm and shape was cubic in nature as shown in fig (1-8). A similar phenomenon has been reported [6, 7].

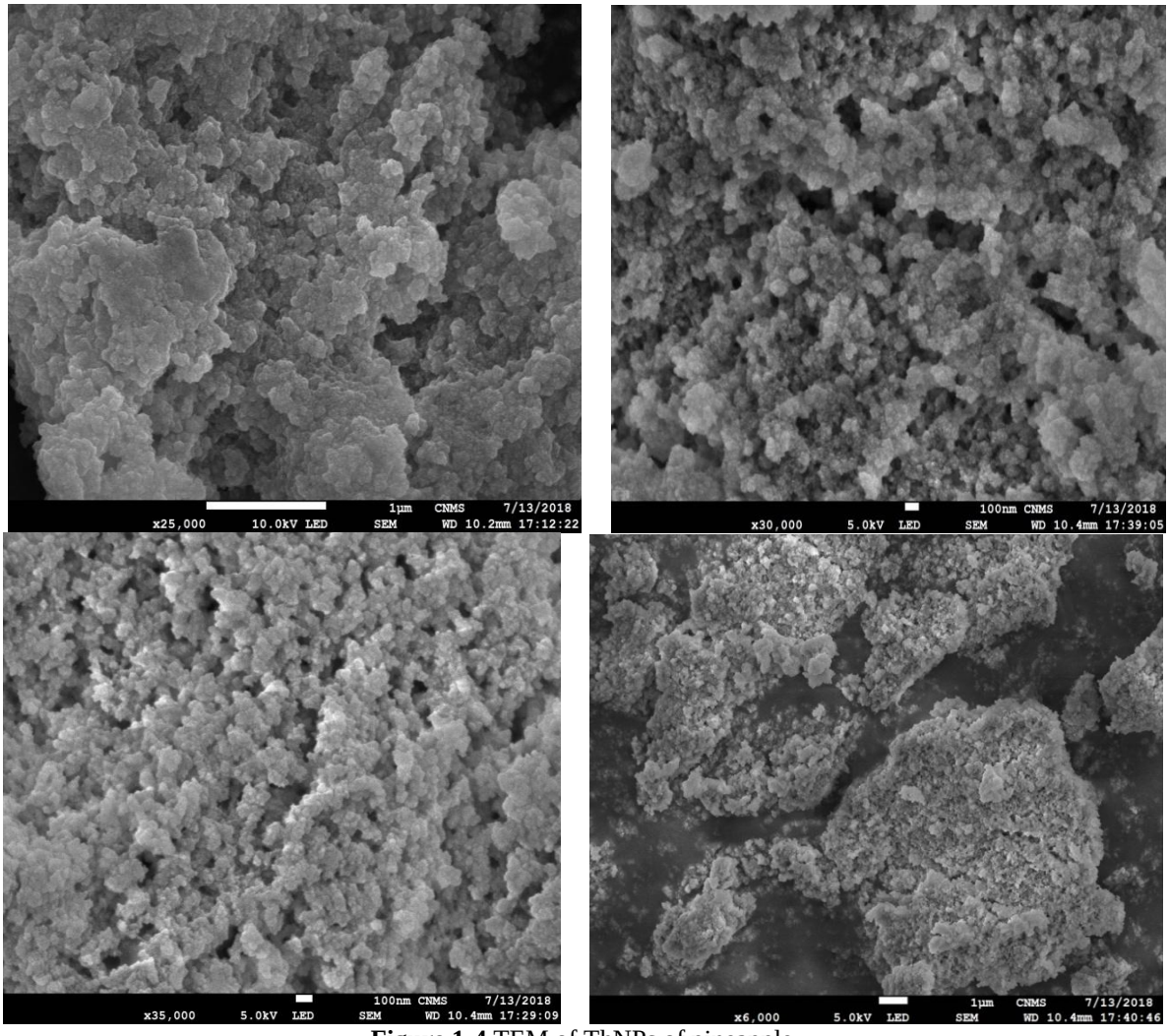

Figure 1-4 TEM of ThNPs of pineapple.

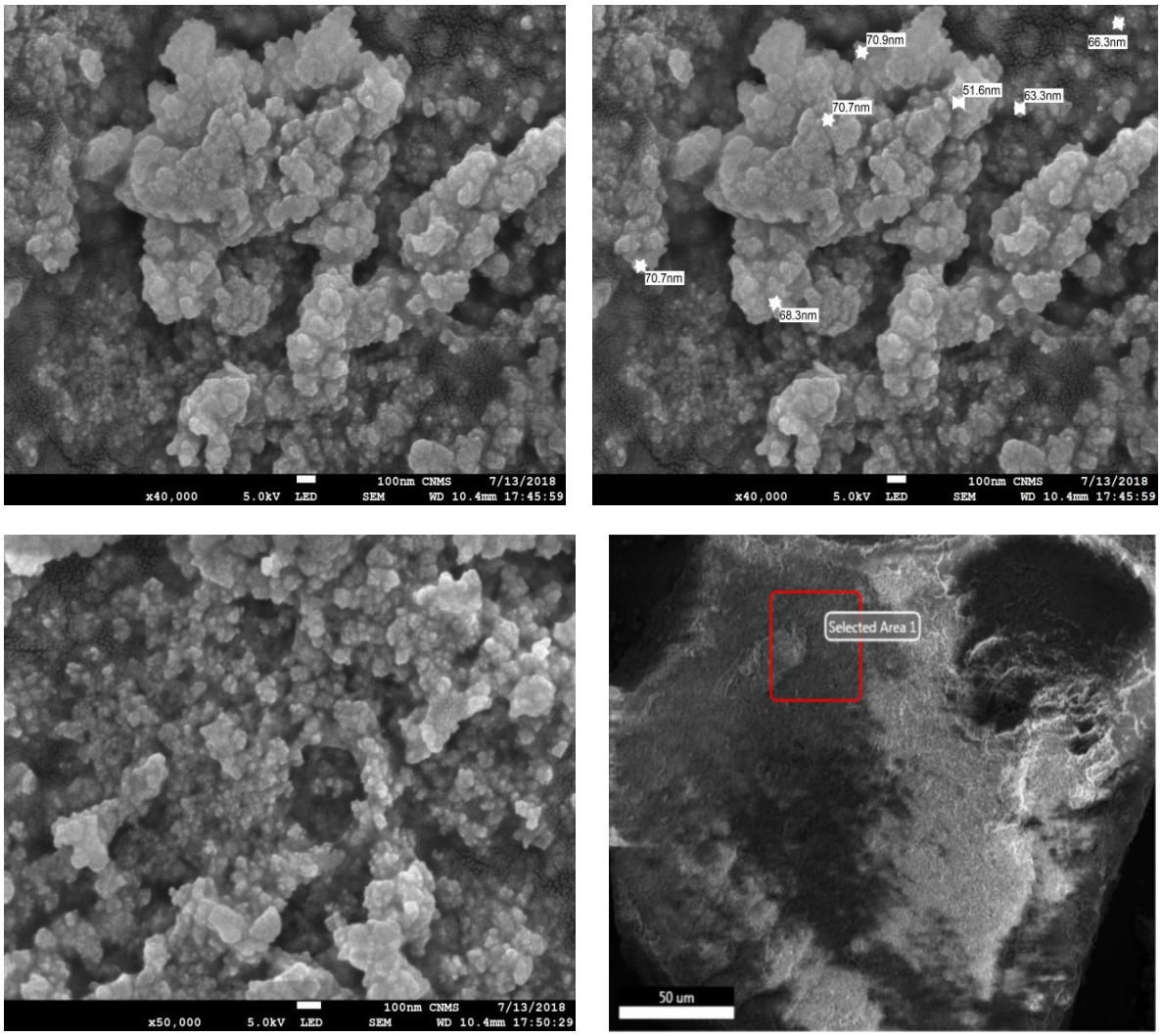

Fig.5-8

Fourier Transform Infra-Red (FTIR) Spectrum

FTIR analysis identified the functional groups responsible for stabilizing ThNPs. Figure 9 represents the FTIR spectra of biosynthesized ThNPs (Blue line) and pineapple extract(Red line). Interestingly, the spectrum patterns of ThNPs and pineapple extract were similar, except for a slight peak shift. For the pineapple extract, the broad peak at 3360 cm^{-1} could be attributed to O-H stretching, while the peak at 2914 cm^{-1} was due to C-H stretching of methyl group. The peak at 16305 cm^{-1} indicated C=O stretching of carbonyl group. The peaks at 1280 and 1050 cm^{-1} were assigned to stretching vibrations of C-N aromatic and aliphatic amines. The general observation showed the presence of the carbohydrates, sugars, proteins and amino acid constituent of the pineapple extract.

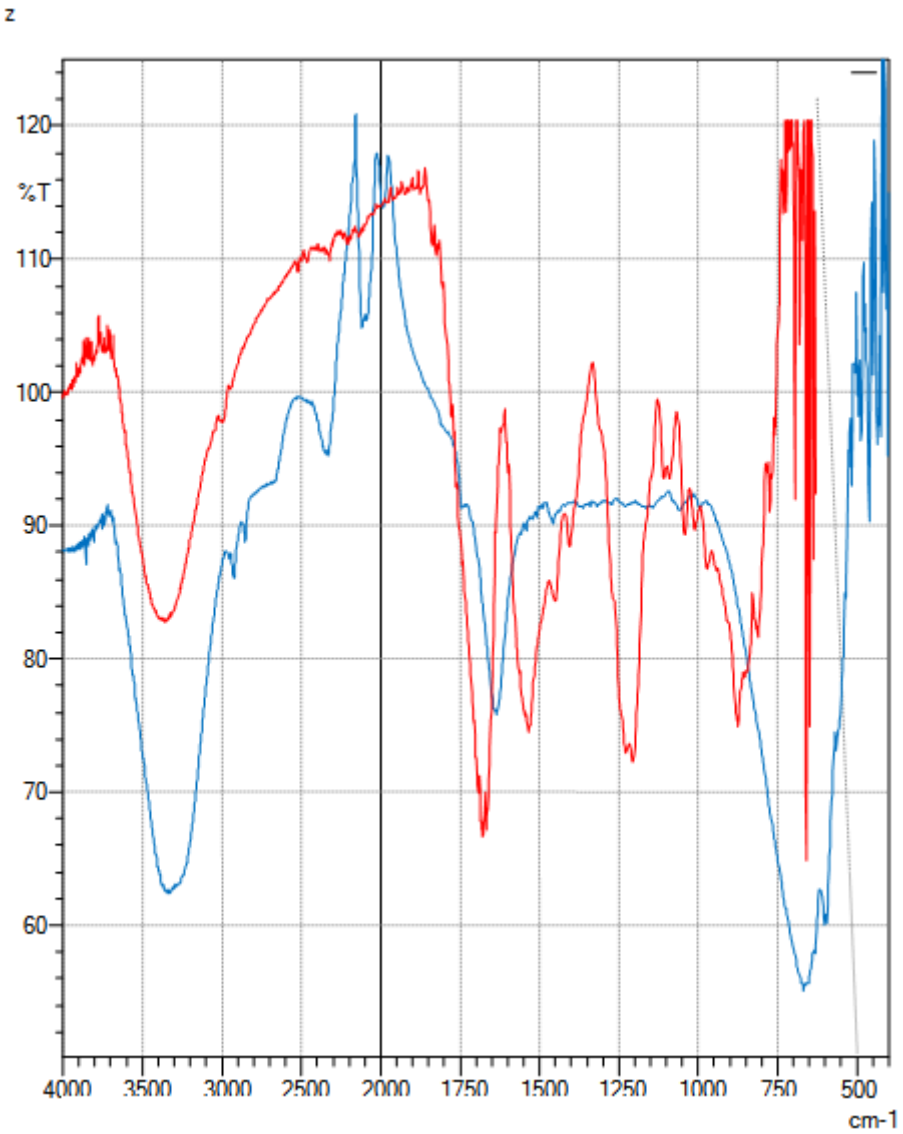

Fig.9. IR spectra (Red line pineapple, Blue line Th NPs)

EDAX Analysis: Energy dispersive spectroscopy (EDAX) of ThO_2 prepared by wet synthesis is shown in Fig.10 which confirms the existence of Th and O with weight percent. EDAX shows peaks of thorium and oxygen of as-prepared sample with less impurity such as

Au and Pd Table.1. The metallic Thorium nanoparticles generally show typical optical absorption peak approximately around 5 KeV due to surface Plasmon resonance.

Table.1

Element	Weight %	Atomic %
Th	35.28	42.19
O	60.08	56.96
Au	3.59	0.28
Pd	2.78	0.4

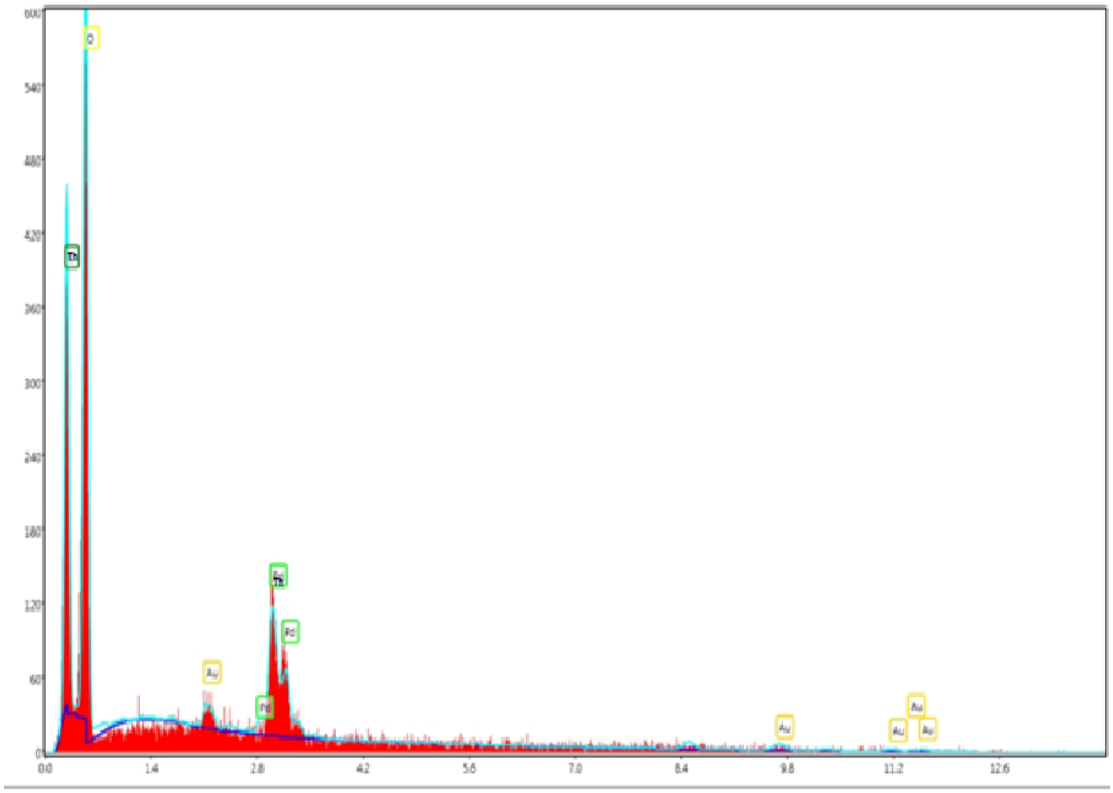

Fig. 10.EDAX spectra of ThNPs

X-Ray Diffraction Analysis:

The PXRD patterns of Th NPs are given in fig.11 PXRD pattern exhibits reflection peaks at 2θ:14.27^0, 31.8^0, 44.46^0 and 57.7^{0,} corresponding to (110), (200), (211) and (220) which shows that sample possesses body centered cubic (BCC) structure. The average crystalline size is determined from Debye-Scherer formula [8]. The crystalline size was determined as 15.98nm.

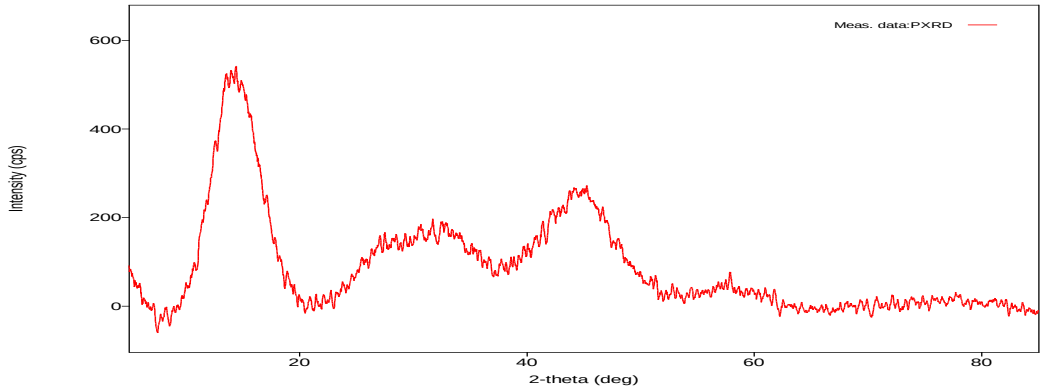

Fig.11. PXRD of ThNPs

Anticancer Activity: - The percentage of viable cells after incubation with test compounds was calculated related to the solvent control (DMSO used for sample preparation). Cell viability results obtained on human melanoma A 375 cell lines are depicted in Fig.9. Determination of A 375 cells viability was performed by MTT assay. Th NPs induced a significant reduction in cell viability of A 375 cells according to a dose and time-dependant manner. The MTT data (fig.12) indicated that, with decrease in concentration the cell viability increases. The current experiment reveals the toxicity of Th NPs on human skin malignant melanoma melanoma (A 375) cells and delivers an important understanding into the probable mechanism by which Th NPs induce its toxic effects on skin cells. Our observations indicate that Th NPs (at 62.5 µg/ml 92.5%) made significant

morphological alterations, which were more significant with increasing exposure time and concentrations of Th Nps and are found to be cytotoxic toward A 375 melanoma cells, their effect appears to bedose-dependant. In addition, there have been scientific reports of the cancer fighting properties of C. Gigantean extract [9]. However, it is known that the uses of medicinal plants have some limitations, such as low bioavailability and stability [10].

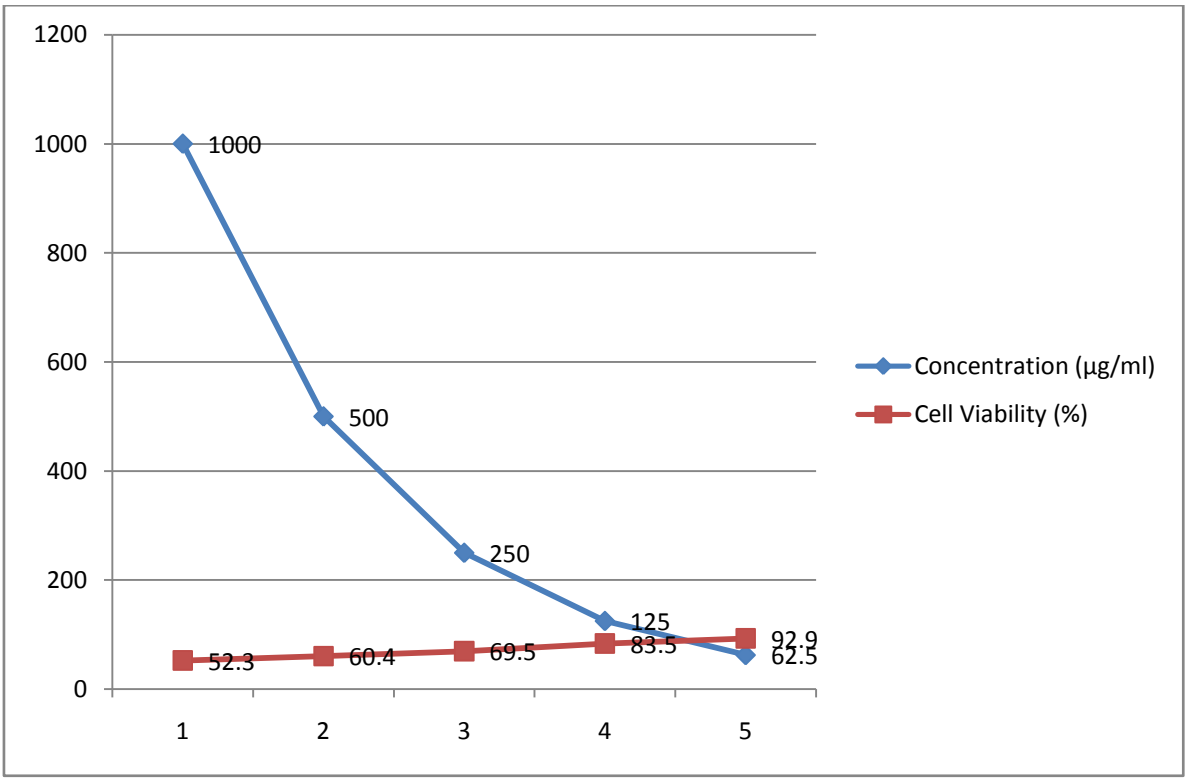

Fig.12. Percentage cell viability due to exposure of Th NPs to human melanoma A 375 cell lines

IV. Conclusion

The Thorium nanoparticles were successfully synthesized using green synthesis method. This method is easy, efficient and eco-friendly, and is the best option for metal. The prepared nanoparticles were characterized to confirm their formation using SEM, FTIR, EDAX and XRD analysis. The in vitro evaluation of the tested samples indicated that pineapple conjugated thorium nanoparticles determined a cytotoxic effect in a dose dependant manner on human melanoma A 375 cell lines and is dependent on concentration. In this study, we have investigated the use of ThNPs to enhance the activity of the pineapple extract on the A375 melanoma cell line. In future, the novel compounds are responsible for the enhancement of such cytotoxic activity will have to be isolated and identified.

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