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GREEN SYNTHESIS AND CHARACTERIZATION OF THORIUM NANOPARTICLES USING ONION (ALLIUM CEPA) EXTRACT AND THEIR ANTICANCER ACTIVITY

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ABSTRACT

The wide application of nanoparticles stimulates the need for synthesizing them, but the conventional methods are usually hazardous and energy consuming. This leads to focus on "Green synthesis" of nanoparticles which seems to be easy efficient and ecofriendly approach. The thorium nanoparticles were characterized by FTIR, UV spectroscopy, Scanning electron microscopy and transmission electron microscopy. Further, the synthesized thorium nanoparticles were tested for antimicrobial and anticancer activity. From the experiment it was found that the thorium nanoparticles synthesized by using onion have a significant anticancer activity. The in vitro results showed that onion coated thorium nanoparticles presented a dose dependant effect on human melanoma A 375 cell lines.

KEYWORDS: Allium Cepa, Nanoparticles,cell line, Anticancer activity.

INTRODUCTION

Nanoscience and nanotechnology have received wide attentions during the last two decades, the base of nanotechnology is to produce the desired nanostructure.[1] Nanoscience and nanotechnology have received wide attentions during the past two decades, where the base of nanotechnology is to produce the desired nanostructures.[1] A nanomaterial is a term of material, which at least possess one dimension in the range of 1-100 nm. Numerous materials with diverse morphology have been prepared. In particular, the metallic nanomaterials have received massive attentions in various areas because of their inherent properties compared to their bulk state.[2] Nanotechnology is a multidisciplinary scientific field undergoing explosive development. The characters of metal and metal oxide nanoparticles have been of great interest due to their distinctive feature such as catalytic activity, optical, magnetic and electrical properties.[3] Nanoparticles interaction with biological materials and established a series of nanoparticle/ biological interfaces that depend on colloidal forces as well as dynamic biophysicochemical interactions. These interactions lead to the formation of new nanomaterial with control size shape, surface chemistry, roughness and surface coatings. With the emergence and increase of microbial organisms resistant to multiple antibiotics and the continuing emphasis on health-care costs, many researchers have tried to develop new, effective antimicrobial reagents free of resistance and cost. Recently, the green chemistry which aims to reduce or eliminate substances hazardous to human health and environment in the design, development and implementation of chemical processes and products is becoming more and more important.[4,5] Nanotechnology is expected to open new avenues to fight and prevent disease using atomic scale tailoring of materials. This is indeed a challenging field of research with unlimited future prospects. Here we report synthesis of thorium nanoparticles in the aqueous solution of thorium nitrate by the help of onion extract. Through elaborate screening process involving number of plants, we observe that onion(Allium cepa) was potential for synthesis of thorium nanoparticles. We also study the antibacterial, antifungal and anticancer activity.

MATERIAL AND METHODS

The onion bulbs were washed with sterile distilled water and the outer covering of the bulb was peeled off and fleshy part of onion was washed with sterile distilled water. The onion bulb was cut into small pieces and 10g of bulb was ground using mortar and pestle with distilled water. The extraction was filtered using muslin cloth and then Whatmann No.1 filter paper. Thorium nitrate (0.1M) was used as precursor for synthesis of thorium nanoparticles. The mixture was incubated at 37^0C. Then the mixture was filtered using watmann filter paper. It was followed by redispersion of the precipitate in deionized water to get rid of any uncoordinated biological molecules.

Characterization of thorium 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).

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.

TEM Analysis of Thorium Nanoparticles: Transmission electron microscopy (TEM) is a microscopy technique where by a beam of electrons is 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 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.

X-Ray Diffraction Analysis: To determine the nature and size of the synthesized ThNPs, X-ray diffraction (XRD) was performed using on an Bruker,D-8 Advance, Germany, which was operated at a voltage of 40 kV and current of 40mA with Cu-Ka radiation.

Anticancer Activity: A 375 cells were subcultured 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 Th NPs (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.

RESULTS AND DISCUSSION

The nanoparticles present a range of characterization challenges that affect the detailed and appropriate characterization of nanoparticles. Nanoparticle characterization is performed to assess the surface area and porosity, pore size, solubility, particle size distribution, aggregation, hydrated surface analysis, shape, size of interactive surface, intercalation and dispersion of nanoparticles and nanotubes in nanocomposite materials.[6]

SEM Analysis

SEM is another technique used to characterize the morphology of nanoparticles through direct visualization. This method is based on electron microscopy and offers several advantages for morphological and size analysis; it is also associated with several disadvantages, such as the ability to provide only limited information about the size distribution and true population average.[7] The visual colour change during the experiment confirmed that Th NPs was successfully generated. The surface morphology of the nanoparticles was characterized using Scanning Electron Microscopy.SEM images of synthesized typical thorium nanoparticles were obtained from Allium cepa extracts and higher images showed that the particles are dispersed and roughly spherical. The particle size (Fig.1,2) of the nanoparticles calculated show that the particles were with sizes of 1-2µm.

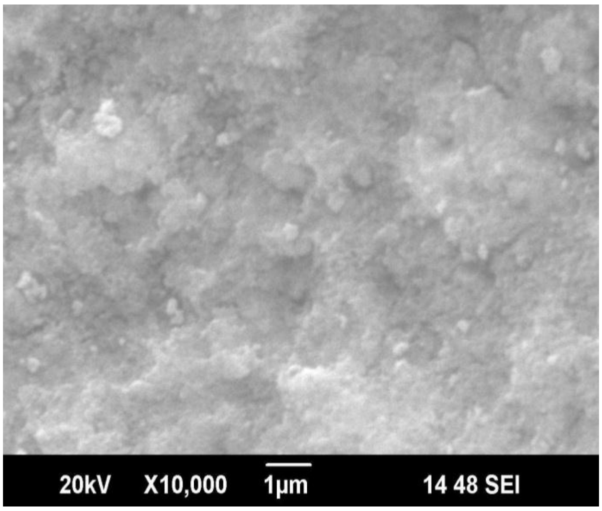


Fig. 1.

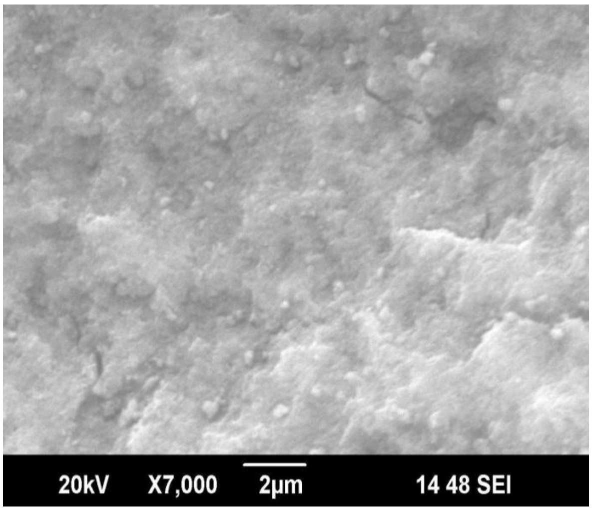


Fig. 2.

TEM Analysis

TEM is one of the most commonly used methods for determination of the shape, size and morphology of nanoparticles.[8,9] The thorium nanoparticles synthesized by the help of onion extract were scanned using TEM from which we can conclude that the average mean size of thorium nanoparticles was 5.69 nm and seems to be spherical in morphology as shown in figure 3,4,5 & 6. Thus the transmission electron microscopy gave a detailed descriptive image of the thorium nanoparticles synthesized with their structural details and their size.

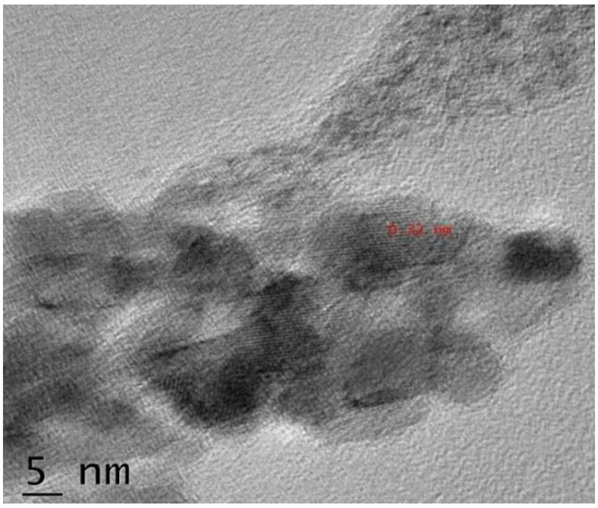


Fig. 3.

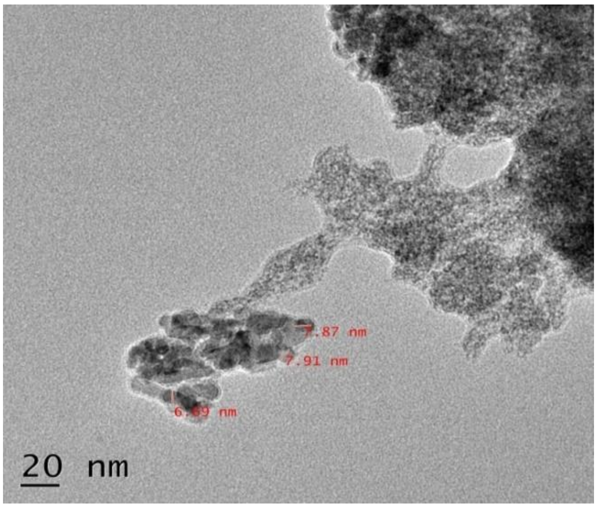
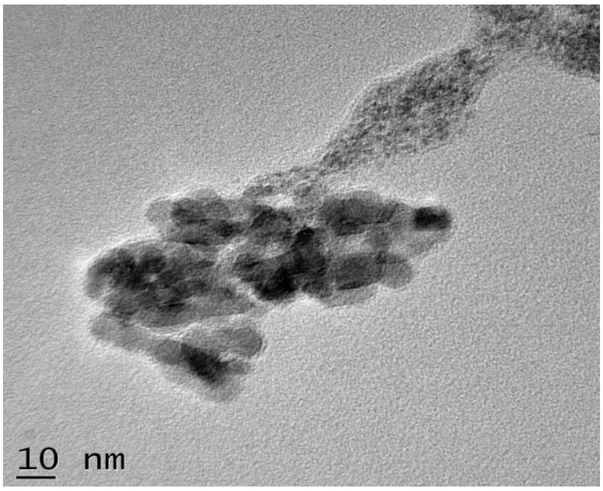


Fig. 4.



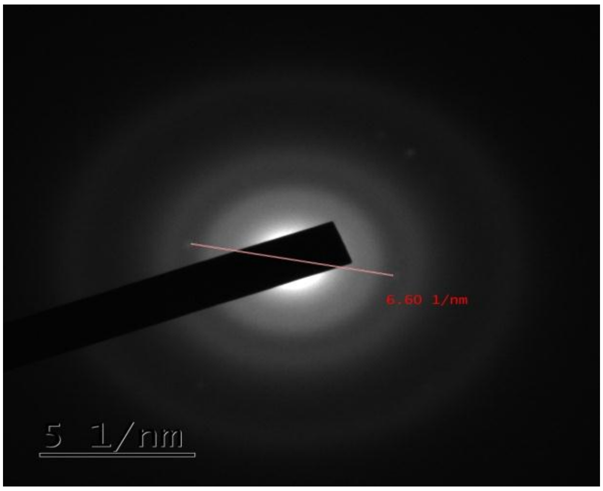


Fig. 5. and Fig. 6. SAED pattern of Thorium nanoparticle.

Fourier Transform Infra-Red (FTIR) Spectrum

The FTIR characterization is used to find the molecules and their functional groups present in the samples. The FTIR spectrum of onion extract showed absorption peaks ranged from 3400 $cm^{-1}$to 400 cm^{-1}.Figure 7 shows the peaks near 3410 cm^{-1}, and 2908 cm^{-1} assigned to OH stretching and aldeydic C-H stretches respectively.[10] After the synthesis of ThNPs, the absorption peaks at 3402 and 1050cm^{-1} corresponding to NH, OH and C-O observed in plant extract get narrower and shifted to higher frequency regions, while those at 2930 and 1658 cm^{-1} corresponding to CH_3 and CO decreases in intensity and shifted to low frequency regions. The structural changes indicated that the reduction and stabilization of thorium nanoparticles proceed via these groups. The bands obtained at 3400 cm^{-1} and between 1700 and 1400 cm^{-1} in the ThNPs spectra corresponding to the amide groups which are the most important band protein infrared spectrum confirm that the phytoconstituents of the plant interact with the thorium nanoparticles. The most useful IR band for the direct measurement of structure of protein is the band found at 1608 cm^{-1} which is related to the maximum absorbance of the amide I of protein.[11] After the reaction, this peak became sharp and decreased in intensity and shifted to 1640 cm^{-1} .Similarly, there is decreased band intensity at 1254 cm^{-1} due to amide III band. This indicated that the structure of the proteins is affected as a consequence of binding with the thorium nanoparticles synthesized from the onion extract are surrounded by some proteins through either free amine groups or cystein residues {12}, and therefore, stabilization of ThNPs by coating of protein is possible.

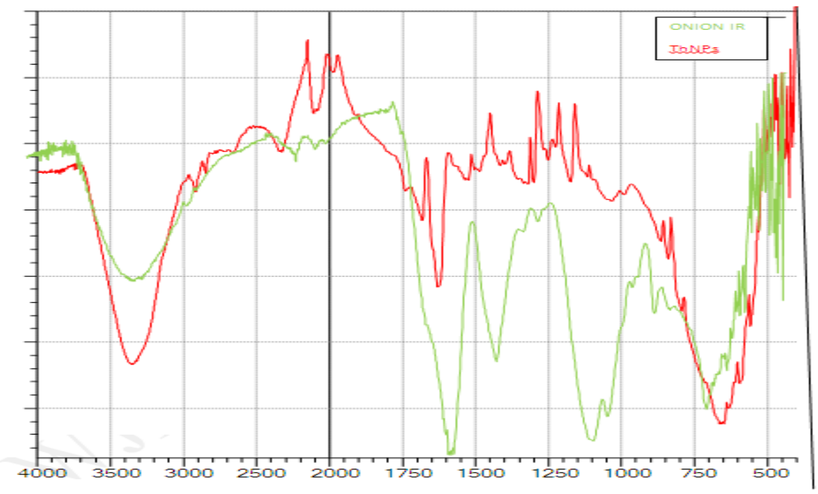


Fig. 7. FTIR spectra of Onion (Green) and Thorium nanoparticles (Red).

X-Ray Diffraction Analysis

The crystal nature of Th NPs studied at room temperature using an X-ray Diffractometer (Bruker,D-8 Advance, Germany). The X-ray source was nickel-filtered CuKα radiation (40 KV, 30 mA). The Th NPs was mounted on a sample holder and scanned in the reflection mode at an angle 2θ over a range of 5 to 60^{o} at a speed of 8^{o}/min. The PXRD pattern of Th NPs obtained from green synthesis were as shown in Figure.8 .The result showed that the structure was in face centered cubic structure. Peaks were absorbed at 16.67^{0},27.85^{0},43.44^{0}alng with miller indices values (111,200 and 220) respectively. As the width of the peak increases size of particle size decreases, which resembles that present material in nano range.[13,14] The average nanocrystalline size calculated from Scherrer formula was found to be 15.70nm.

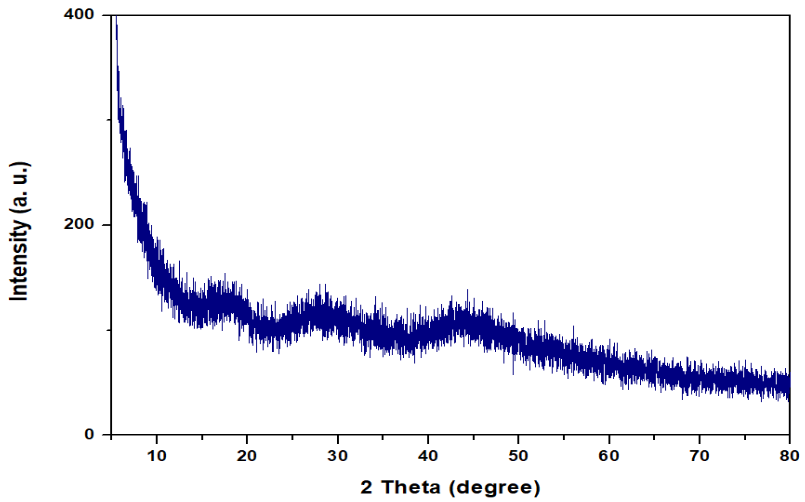


Figure. 8. XRD Pattern of Th NPs.

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. 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.9) 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 58.7%) made significant morphological alterations, which were more significant with increasing exposure time and concentrations of Th Nps.

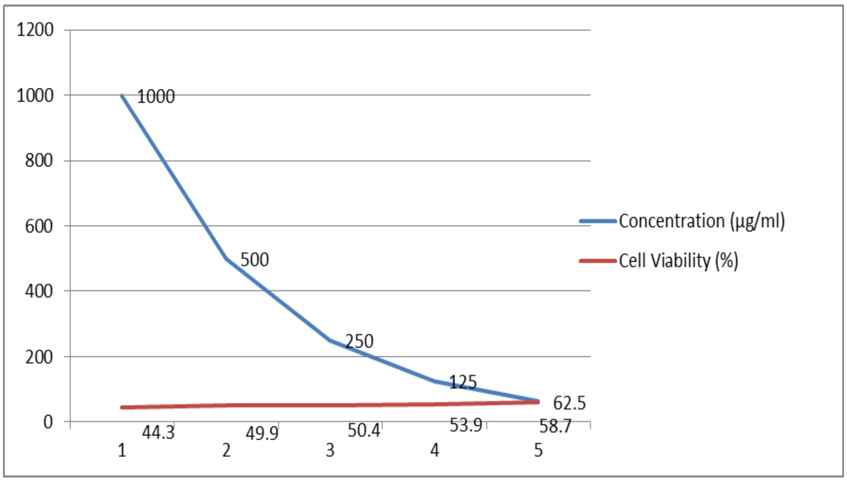


Fig. 9. Percentage cell viability due to exposure of Th NPs to human melanoma A 375 cell lines.

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, TEM, FTIR and XRD analysis. The in vitro evaluation of the tested samples indicated that onion conjugated thorium nanoparticles determined a cytotoxic effect in a dose dependant manner on human melanoma A 375 cell lines and is dependent on concentration.

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