Synthesis and Characterization of Thorium nanoparticles using Brassica Oleracea (Cabbage) extract and anticancer activity

*Mahantesh.Kumbar Vanita. Dyavannavar, *,Savita Akale, and Mallikarjun S.Yadawe*

S.B.Arts and K.C. P Science College, Vijayapur, Karnataka India.

Abstract: - The objective of this study was the synthesis of thorium nanoparticles using cabbage extract as the reducing and stabilizing agent. The formation of nanoparticles was initially confirmed by the colour change and by transmission electron microscope (TEM).The size of ThNPs ranged from 5-100nm.Variuos instrumental techniques were adopted to characterized ThNPs, viz FTIR, XRD and anticancer activity. ThNPs could be a brilliant candidate to be used as a chemotherapeutic agent against invasive breast cancer cells.

Key words: - Cabbage, synthesis, nanoparticles, TEM, XRD, FTIR and anticancer activity.

Introduction:-Nature has devised various processes for the synthesis of nano and micro length scaled inorganic materials which have contributed in the development of relatively new and largely unexplored area of research based on the biosynthesis of the nanomaterials. Synthesis is using bio-organisms is compatible with the green chemistry principles. “Green synthesis “of nanoparticles makes use of environmental friendly, non-toxic safe reagents. Nanoparticles synthesized using biological techniques or green technologies have diverse natures with greater stability and appropriate dimensions since they are synthesized using a one-step procedures.

Chemical, physical and biological methods have been developed to synthesis nanoparticles but chemical and physical methods are involved in the production of toxic byproducts which are hazardous moreover the methods are very expensive [1, 2].Plants provide a better platform for nanoparticles synthesis as they are free from toxic chemicals as well as contain natural capping agents [3].Nanotechnology application is now widely distributed throughout life and especially in agricultural systems. Nanoparticles, because of their physiological characteristics, are among the potential candidates for modulating the redox status and changing the seed germination, growth, performance and quality of plants [4]. The numerous studies have reported the effects of NPs on seed germination and crops improvement. However, most of the experiments have been done under controlled conditions. Studies indicate that carbon nanotubes (CNTs) penetrated tomato seeds and dramatically enhanced their germination percentage and growth parameters [5].

The vegetable cabbage is used to synthesis the thorium nanoparticles. These are the vegetables of the Brassicaceae family. It is originally from Europe and Asia. These vegetables have potent biological and immunological activities. It also has various chemical constituents. They are very good source of electrolytes, minerals, vitamins and dietary fibers, isothiocyanate antioxidant compound called sulforaphane [6]. Even

though ThNPs have been syntheisized from various biological sources, the synthesis of ThNPs from cabbage is not much reported in literature. So this investigation is designed to synthesis the ThNPs from cabbage and to evaluate the anticancer activity.

Materials and Methods

Synthesis of Thorium Nanoparticle

Cabbage vegetables were purchased from the Vijayapur local market. The extraction sample was prepared by extracting the juice of the leaves, sieving it and storing it for the synthesis of ThNPs. Both fresh and refrigerated extract were used and they yielded similar results. An aqueous solution of 0.1M thorium nitrate was prepared. Cabbage extract 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.

TEM Analysis of Thorium 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(IISc Bangalore).

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 Xpert Pro MPD, which was operated at a voltage of 40 kV and current of 40mA with Cu-Ka radiation.

Anticancer activity:-

Cell culture:- 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), pencillin and streptomycin (both from Sigma) in a 50% carbon dioxide (CO_2) cell incubator at 37^OC 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 $1X10^4$ cells/well for analysis [11, 12].

Results and Discussion

Characterization of synthesized thorium nanoparticles

TEM Analysis: - Figure 1-5 show representative TEM images of obtained ThNPs. The nanoparticles of smaller diameter can easily pass through the membrane channel of the bacteria [7]. The TEM image of the synthesized thorium nanoparticles shows they are spherical shape with a smooth surface morphology. The diameter of the nanoparticles is found to be in the range of 5nm to 100nm and analyzed that the produced nanoparticles are more or less uniform in size and shape.

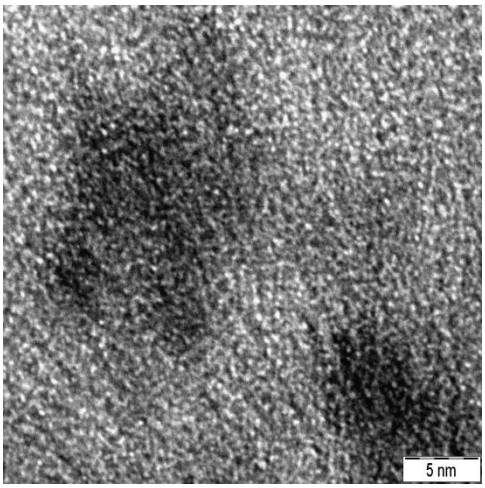

Fig.1

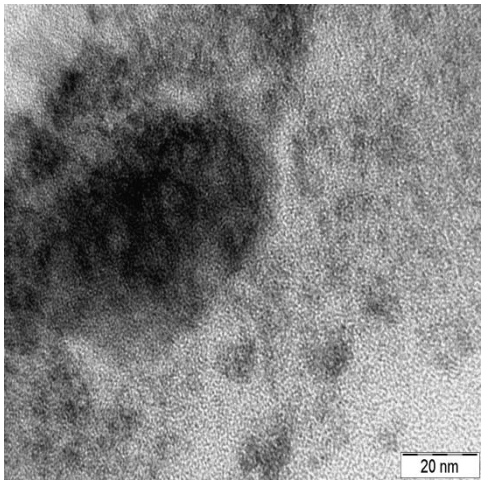

Fig.2

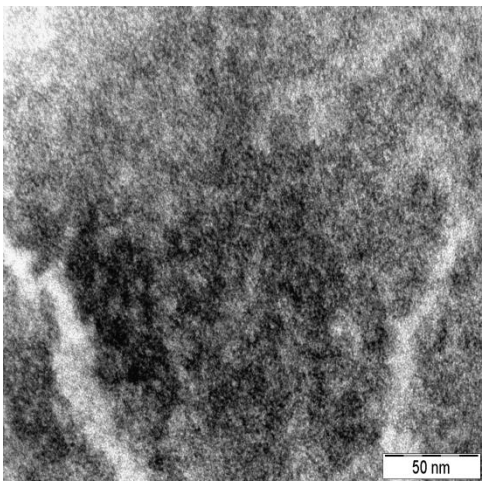

Fig.3

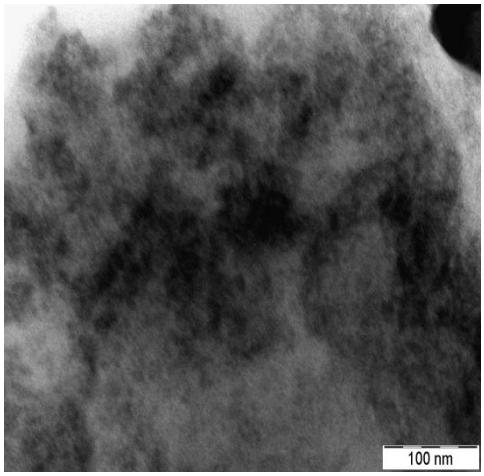

Fig.4

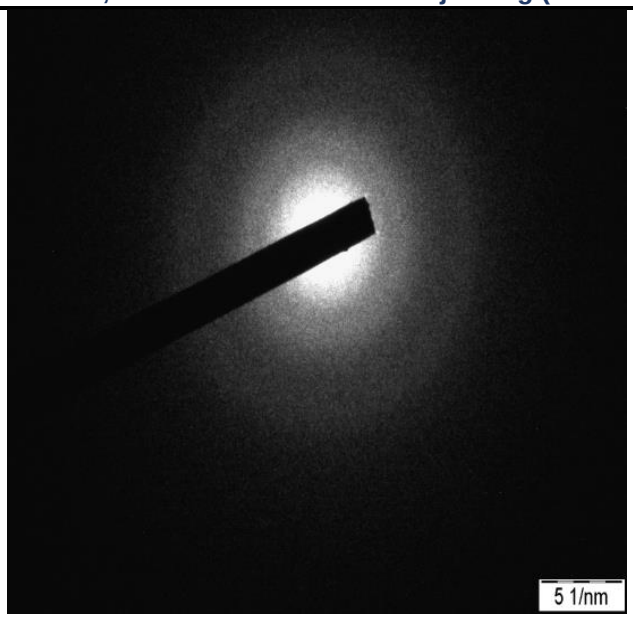

Fig.5. Selected area electron diffraction (SAED)

Fourier Transform Infrared spectroscopy of ThNPs:- The FTIR spectrum of synthesized ThNPs from cabbage extract and cabbage have been shown in fig.6. The band at 3450 cm^{-1} and 3445 cm^{-1} corresponds to O-H stretching H-bonded alcohols and phenols. The peaks at 2360 cm^{-1} and 2350 cm^{-1} corresponds to C-N stretching bonds of nitriles. The peak at 2180 cm^{-1} and 2020 cm^{-1} corresponds to aromatic –CH stretching bonds. The band at 1650 cm^{-1}corresponds to N-H band primary amines corresponding to amide I arising due to carbonyl stretching in proteins. The band at 1405 cm^{-1}coresonds to aromatic rings. The presence of peak at 1030 cm^{-1} relates with the $^{\upsilon}$(C-O-C) of glycosidic linkage belonging to the plant [8]. The peak at 680 cm^{-1} and 660 cm^{-1} corresponds to C-H bend of alkynes. The corresponding values have confirmed the fact that the carbonyl group form amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could be possibly form a layer covering the metal nanoparticles (i.e capping of thorium nanoparticles) to prevent agglomeration and thereby stabilize the medium[9].

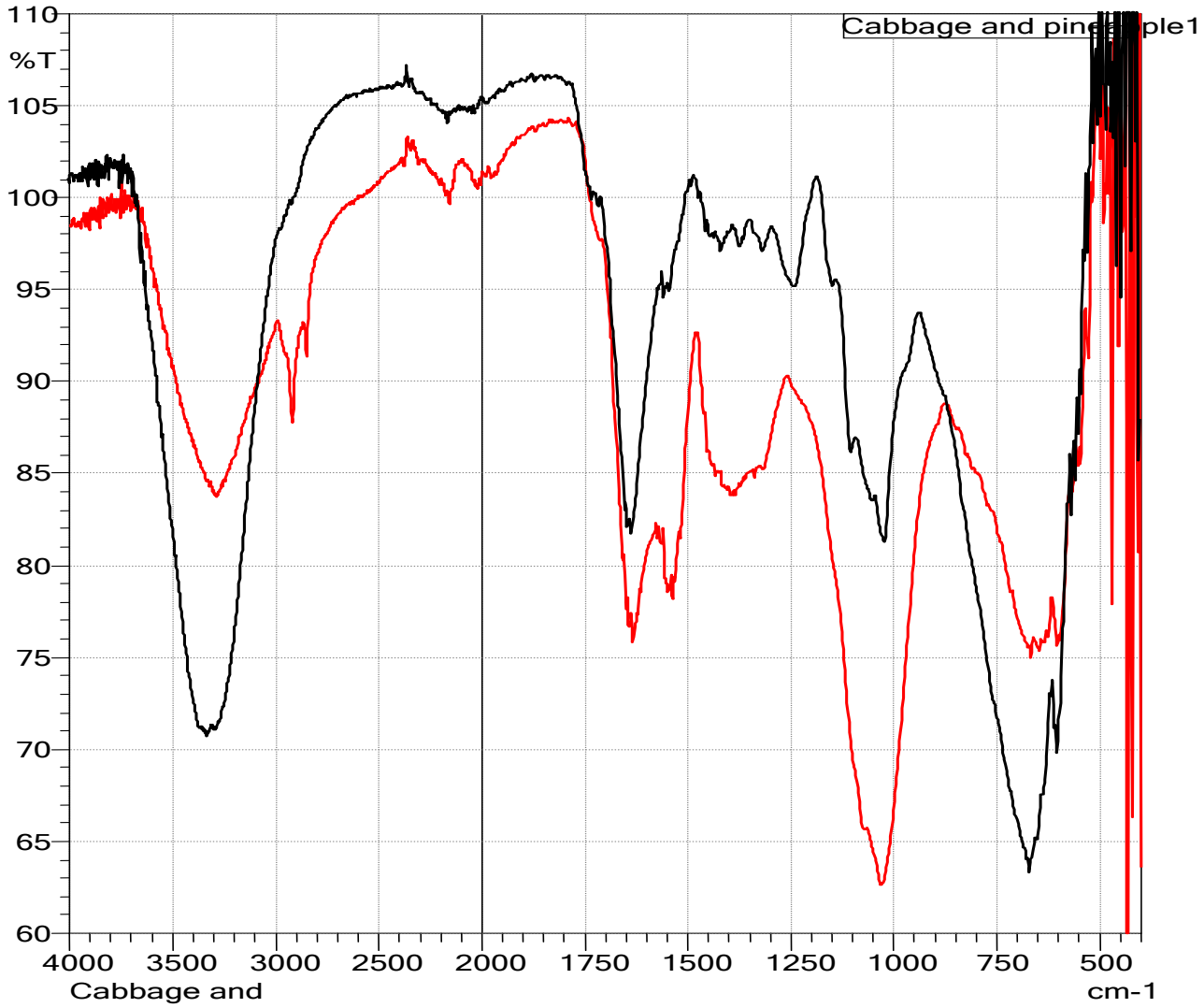

Fig.6

X-Ray Diffraction Analysis:-Powder XRD is used to investigate the phase structure and crystalline size of the synthesized nanoparticles. XRD pattern of the Thorim nanoparticles with cabbage is shown in fig.7. It is found that all the major diffraction peaks can be can be indexed with standard thorium nanoparticles with face centered cubic. The diffraction peaks observed at 2 values 38.06, 44.53, 58.55, 68.54 are attributed to face centered cubic structure(JPCDS-35-0103.The average crystalline size is determined from the Debye-Sherrer formula.The crystalline size is determined as 16.20nm. The morphology of the product was further characterized by SEM.

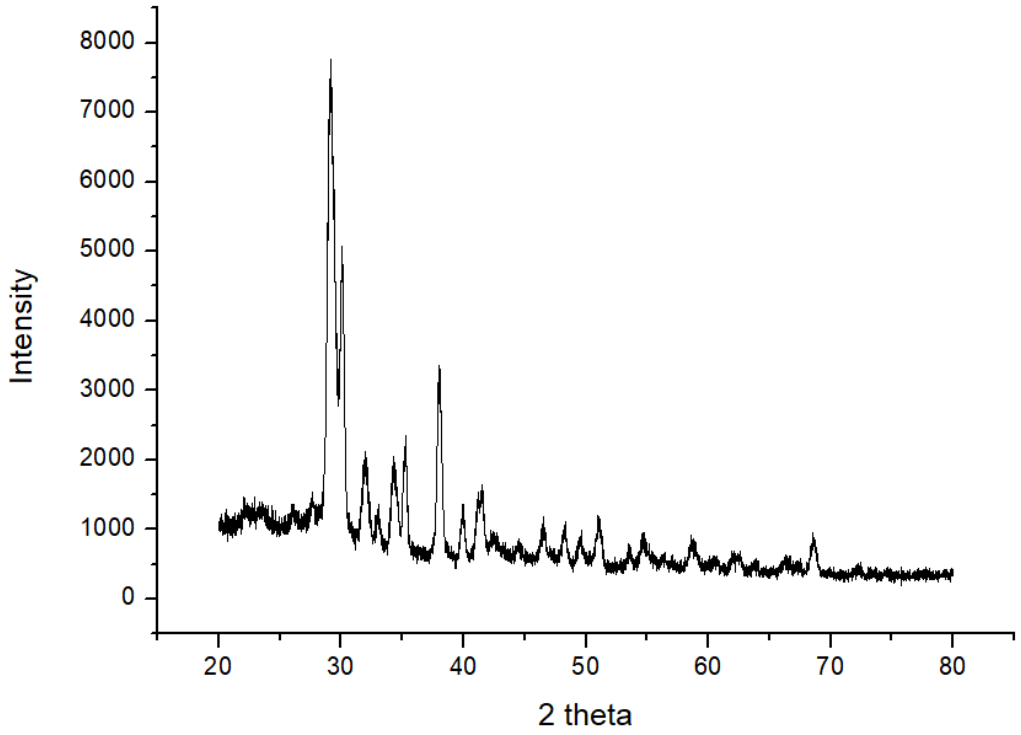

Fig.7

Anticancer Activity:-

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

Table.No.1. Anticancer activity cabbage naonoparticles with ThNPs 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 µg/ml) of cabbage naonoparticles with Thorium are tabulated in Table 1. MTT assay of cabbage naonoparticles with ThNPs demonstrated significant growth inhibition in human breast cancer cell line [MDA-MB-231]. The percentage of cell viability was found to be 83.33, 66.66, 60.62, 53.12 and 44.16 respectively at concentrations of 62.5, 125, 250, 500 and 1000 µg/ml of test compound.

Table.No.1.

Compound	Concentration (ug/ml)	Cell Viability (%)
Cabbage Naonoparticles with Thorium	1000	44.16
	500	53.12
	250	60.62
	125	66.66
	62.5	83.33

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