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BIOSYNTHESIS OF ZINC OXIDE NANOPARTICLES USING ORANGE PEELS EXTRACT FOR BIOLOGICAL APPLICATIONS

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ABSTRACT

The synthesis of zinc oxide nanoparticles from the orange peel is a useful and ecofriendly method alternative than using chemical processes, due to the pollution mechanism and the risks that accompany chemical processes. Nanoparticles have been diagnosed by several techniques, including Fourier transform infrared spectroscopy (FTIR) is used for analyzing the functional groups, Atomic Force Microscope (AFM), scanning electron microscopy (SEM) morphological studies and X-Ray Diffraction (XRD) to analyze the structure of crystalline materials And knowledge of its medical applications through its application to serum patients who have high thyroxine.
Keywords: Green Synthesis, ZnO nanoparticles, Thyroxine

Introduction

The area of nanotechnology it is considered the most range active areas of research in modern material (Ambrosi *et al*., 2009). The word nano is taken from the Greek language and gives the meaning of short man, Nanotechnology involves the characterization of materials that have at three dimension or zero dimension, Their scale is approximately from 1-100 nm in length the metallic nanoparticles one has It has many important fields such as electronics, cosmetics, coating and biotechnology (Asmathunisha *et al*., 2013). Nanoparticles are classified into major types, viz., organic and inorganic (Bar *et al*., 2009). All this ways based on top – down and bottom – up techniques (Castro *et al*., 2011). Green nanotechnology refers to the use of nanotechnology to enhance the Environmental safety (Gadekar *et al*., 2014) and is today ushering in a new era in the furtherance of science and technology (Jalill *et al*., 2016).

Lately, numerous plant extracts have been shown to be innovative resources for their capability to produce safe and nontoxic Nanoparticles (Kuppusamy *et al*., 2016). The methods involved are typically simple, environmentally friendly and naturally compatible one-pot processes (Portal *et al*., 2010). Plant extracts are bioactive polyphenols, proteins, phenolic acids, alkaloids, sugars, terpenoids (Premanathan *et al*., 2011) etc. Plant-mediated synthesis of metal nanoparticles has become very popular due to ease and availability of plant material which contain reducing compounds such as amino acids, enzymes, flavonoids, sugars, aldehydes, ketones, amines, carboxylic acids, phenols, proteins, alkaloids, terpenoids, pigments and other reducing agents present in the plant extracts and microbial cells (Singh *et al*., 2011).

The mechanism of nanoparticles depends on many variables, such as pH, concentration of bilogy particles in plant extract or in brine, incubation time and temperature (Singh *et al*., 2011). The exact mechanism of nanoparticles synthesis by biological extracts is yet to be understood, this is because each biological molecule is taken to a different mechanism to interact with the ion solution (Hulkoti *et al*., 2014). Reduction and stabilization of nanoparticles are attributed to the phenolic and/or hydroxyl-substituted functional groups (Dwivedi *et al*., 2015).

Tetraiodothyronine (T4), a form of thyroid Hormones, it is one of the most important thyroid hormones that plays a major role in controlling metabolism by controlling protein synthesis through DNA. Thyroxine is synthesized via the iodination and covalent bonding of the phenyl portions of tyrosine residues, the half-life of T4 once released into the blood circulatory system is about one week (Magner *et al*., 2014).

In the present study, anovel biomimetic synthesis of biosynthesized zinc oxide nanoparticles in the presence of orange peels extract has been studiedat room temperature. This is particularly advantageous because this method utilizes orange peels wasteas a resource material as well as a natural ligation agent for innovative biomedical applications. Moreover, the orange fruit peels contain powerful phenolic antioxidantsare responsible for the potential utilization in both food and medical industry. Therefore, we explored whether the formed ZnONPs had enhanced biomedical applications. We found that the ZnONPs had enhanced the free hormone thyroxine when compared to the control.

Materials and Methods

Preparation of Orange Peel Extract

The collected orange peels were rinsed in double distilled water and then air-dried and finally grained to fine powder. Ten grams of finely powdered peels were extracted in a water bath containing 250 ml of deionized water. Then, the solution was refluxed for 1hr. The extract was filtered through Whatman filter paper and finely collected for used to synthesized zinc oxide nanoparticles.

Synthesis of zinc oxideNanoparticles

Zinc nitrate (Zn $(NO_3)_2.6H_2O$) was used for the synthesis of zinc oxide nanoparticles. 2.5 g of zinc nitrate were added in 100 ml deionized water, after that peels extract were mixed with it in separated flask. Zinc nitrate solution was reduced to zinc oxide, which was indicated by a color change. Then the solution was subjected to continuous stirring at 100 rpm for 4 hr. The pH of the solution was maintained 12 by drop wise addition of sodium carbonate solution. After that the solution is heated to 80 ^{o}C for half an hour. The resultant nanoparticle were settled in the bottom of the flask, and after that the solution was purified by centrifugation at 5000 rpm/min for 5 min. Finely, the zinc oxide nanoparticles were filtered and air dried over night using Alpha 1–2 LD plus and stored.

Biological Application

This study included thirty healthy people, their ages ranged between (9-60) years. Specimens were collected during the period from 1st Nov 2019 until the end of Feb 2020. Venous blood samples (4-5 ml) have been collected from each individual of healthy control between 8AM and 11AM. The serum collected by putting the blood in a clean and dry gel tube and left to clot at 37 ºC for 20 min, then put it in the centrifuge device at 6000 RPM for 10 min, serum are separated in a simple plastic tube and kept in the freezer till used for the assay. Biological experiment was done by adding 10 microns of serum to 10 micro liters of colloidal nanoparticles in concentrations (0, 25, 50, 100, 150, 175, 200) ppm. The free T4 assay is a two-step immunoassay anti-free T4 coated nanoparticles are combined with the free T4 present in the sample. After washing, T4 acridinium-labeled conjugate is added to create a reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs) by using Abbott device.

Atomic Force microscopy (AFM)

The surface characterization and particle size examination of zinc oxide nanoparticles were carried out by Atomic Force Microscopy (AFM), Angstrom Advanced Inc., England.

X-ray diffraction (XRD)

X-ray diffraction (XRD) was used to confirm the crystal structure (crystallite size and crystal phases) of zinc oxide Nanoparticles. XRD analysis were carried out using Shimadzu X-ray diffractometer (Japan) with Cu crystal radiation (λ = 1.5418 Å) scanning at a scan speed of 8.0000 deg/min for drive axis is theta-2theta and scan range 10.0000 - 120.0000 deg. The estimation of the sizes of particles was performed by Debye–Scherer's formula.

Fourier transforms infrared spectroscopy (FTIR)

The spectra properties of zinc oxide Nanoparticles were observed by Fourier transform infrared spectroscopy (FTIR) analysis using the dried powder of the synthesized zinc oxide nanoparticles by FTIR spectrometer Shimadzu, IR Affinity 1, Japan. The pellets were scanned at 4 cm^{-1} resolution in the spectra range of 4000–500 cm^{-1} at room temperature.

Scanning electron microscopy (SEM)

The structural morphology of zinc oxide nanoparticles was examined and measured by Scanning Electron Microscopic (SEM) using Tuscan, Hungary. An aliquot of each sample was fixed on a carbon-coated copper grid, and the film on the SEM grid was then dried by fixing it under a mercury lamp for 5 min. The instrument was equipped with an energy dispersive spectrum (EDS) to ensure the presence of nanoparticles.

Results and Discussion

Characterization of Orange peels nanoparticles

Pale white colored zinc oxide nanoparticles were obtained by green synthesis method using zinc nitrate ($Zn(NO_3)_2.6H_2O$), sodium carbonate and orange peel extract. The synthesized nanoparticles were characterized by different analytical techniques such as AFM, XRD, FTIR, SEM and EDX. Synthesis of nanoparticles with orange peels extract is a novel approach which is affordable and eco-friendly. The phytochemical analysis of orange peels show that the main constituents of peels were tannins, saponins, phenols, terpenoids, alkaloids and flavonoids (Edori *et al.*, 2018). The reaction mechanism for the formation of zinc oxide nanoparticles is as a result of the reaction of the zinc ions present in the solution with the phenols and polyphones. Such as the tannins, terpenoids, and flavonoids present in the orange peels extract forming complexation. This is followed by hydrolysis reaction to form zinc hydroxide due to the presence of the hydroxyl group in the phenols. Calcination and decomposition reaction shortly follows to form zinc oxide Nanoparticles. Also, the synergistic reaction of all the phytochemical components present in the extracts act as both reducing and stabilizing agent by reducing zinc to the zero valence state (Basnet *et al.*, 2018). The synthesis of zinc oxide was used for further characterization.

Morphological of AFM indicate that green synthesized zinc oxide nanoparticles produces topological images of surfaces at a very high magnification and facilitates the observation of the atomic structure of crystals. Figure 1 show two dimension image indicating that zinc oxide nanoparticles had an average diameters of 71.06 nm.

The X-ray diffraction (XRD) graph showed peaks only due to ZnO (Figure 2). synthesized zinc oxide nanoparticles showed strong diffraction peaks at 36.3, 31.8, 34.4, 47.8, 56.7, 63.1, 66.2 and 68.1 degrees of 2. The ecofriendly synthesized zinc oxide nanoparticles diffraction peaks corresponded to (001), (002), (101), (102), (110), (103), (200) and (112) crystal planes. The narrow and strong diffraction peaks indicate the well crystalline nature of zinc oxide. The size of ZnO nanoparticles was obtained by Debye–Scherer's formula given by the equation (Vijayalakshmi *et al.*, 2012):

$$D = k \lambda / (\beta \cos \theta)$$

where D is the crystal size, λ is the wavelength of the X-ray radiation (λ = 0.15406 nm) for CuKa, K is usually taken as 0.89, β is the line width at half-maximum height (17). The Scherrer's formula was used to calculate the particle sizes and was found to be in the range of 40-50 nm. XRD study confirmed the presence of smaller particles than the SEM examination. The large nanoparticles of ZnO in the sample result from the agglomeration of smaller nanoparticles (figure 4), whose presence is confirmed by X-ray diffraction . The XRD method allowed for the identification of smaller sizes of nanoparticles. The agglomeration of smaller nanoparticles occurs due to the fact that we are dealing with biological material.

The main bands and corresponding assignments common to the synthesized ZnONPs were as follows: 3372 cm^{-1} and 3178 cm^{-1} (broad bands of the O-H stretching vibration), 1656 cm^{-1} and 1427 cm^{-1} (C=C stretch and C-C stretching respectively) while 545 cm^{-1} was characteristic bands of the zinc oxide nanoparticles (Figure 3). The FTIR spectrum, absorption at 400 cm^{-1} to 600 cm^{-1} identifies the presence of zinc oxide nanoparticles (Yuvakkumar *et al.*, 2015) which further confirms the formation of zinc oxide nanoparticles by using orange peels.

SEM images of the obtained for synthesized zinc oxide nanoparticles were agglomerated with a particle size ranging from 100 - 200 nm. To gain further insight into the features of ZnO nanoparticles, the analysis of the sample was performed using EDS technique. The energy dispersive spectra of the samples obtained from the SEM-EDX analysis show that the sample prepared by the above route has pure ZnO phases (Kumar *et al.*, 2013). The EDS studies of Figure 3 present three peaks between 1 kV and 10 kV. Those maxima are directly related to zinc in the tested material. The results indicated that the reaction product was composed of high purity zinc nanoparticles. Additionally, the presence of highly pure ZnO is confirmed by X-ray diffraction XRD (Figure 2).

Effect of ZnO Nanoparticles on free thyroxin FT4

Figure (5) shows the effect of ZnO NPs on free thyroxin, when reviewing the results, it was found that the effect of nanoparticles is high at a concentration of 25 ppm. From this inhibition effect, nanoparticles of zinc oxide can be used as a pharmaceutical compound. This inhibition is explained by the hydrogen bonds between the acidic side chains of the amino acids containing hydroxyl or an amine groups.

Conclusions

It is well known that the green synthesis of zinc oxide nanoparticles is much safer and environmentally friendly as compared to chemical synthesis. In response to this assumption, this study demonstrates the green synthesis of zinc oxide nanoparticles using orange peels water extract. In this study, zinc oxide nanoparticles were successfully biosynthesized and clarifying the role played by the biomolecules present in orange peels, in the reduction and stability present in the formation mechanism. The resultant nanoparticles were characterized using FTIR, XRD, SEM, TEM, and EDS. Effect of the ZnONPs were examined on thyroxin in thyroid disease and it was found that the effect of zinc oxide nanoparticles on the free hormone thyroxine by reducing its in serum, which explains to its pharmacological value.

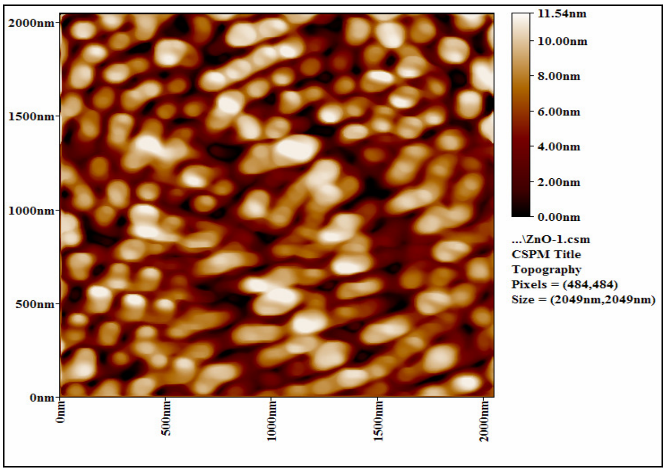

Fig. 1: Atomic Force Microscope for ZnO NPs

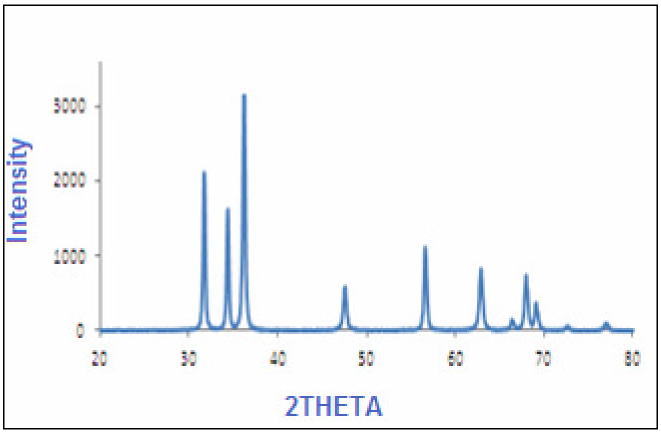

Fig. 2 : X-ray diffraction for ZnO NPs

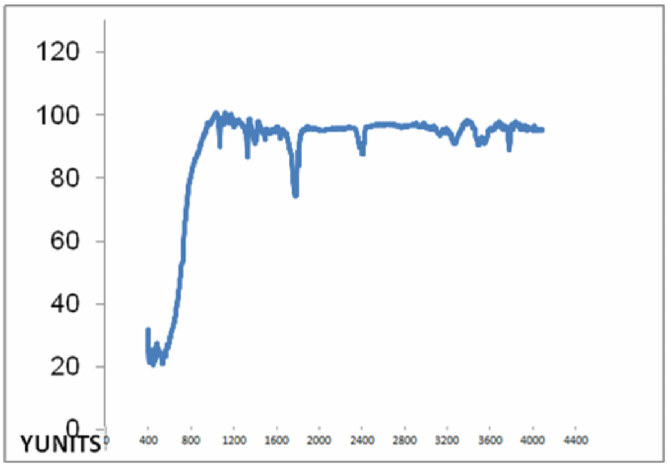

Fig. 3 : FTIR spectra of ZnO NPs

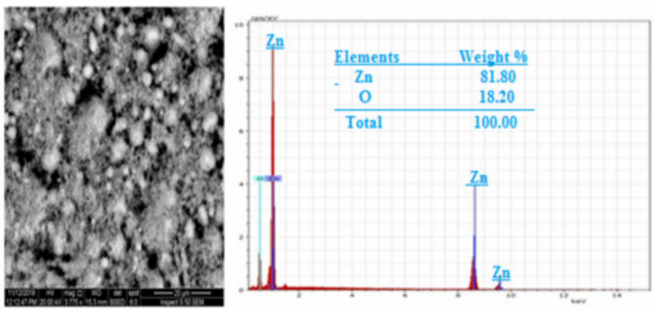

Fig. 4 : SEM and EDX of ZnO NPs

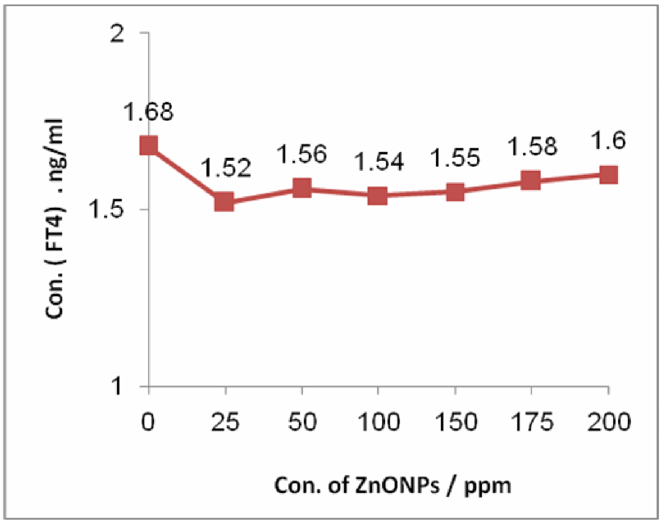

Fig. 5 : Effect of ZnO NPs on FT4

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