Anticolon Cancer Activity of Zinc Oxide Nanoparticles Using Fresh Leaf Extract Nyctanthes Arbor-tristis

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Research Article

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Anticolon cancer activity of Zinc Oxide Nanoparticles Using Fresh Leaf Extract *Nyctanthes arbor-tristis*

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Abstract

The present study reports the green synthesis of Zinc Oxide nanoparticles using the aqueous leaf extract *Nyctanthes arbor-tristisis* by co-precipitation method. The synthesized zinc oxide nanoparticles are characterized by X-Ray diffraction (XRD), Fourier Transform Infrared Spectral Analysis (FTIR), UV-Visible spectroscopy (UV-Vis), Scanning Electron Microscopy (SEM) and Energy dispersive X-Ray Analysis (EDX). The Zinc Oxide nanoparticle are crystalline in nature and have hexagonal structure with the particle size of about 25 nm and is determined by XRD analysis. The capping agent and the functional groups of the Zinc Oxide nanoparticles are determined by FTIR spectral analysis. The band gap energy is about 3.18 eV for the synthesized ZnO nanoparticles which is calculated by UV-Visible spectroscopy. The surface morphological structure of ZnO nanoparticles is spherical in shape. The EDX spectroscopy is used to determine elemental composition of ZnO nanoparticles. The anticancer activity of Zinc Oxide nanoparticles have high toxicity against HT-29 cell line which is determined by MTT assay. The synthesized ZnO nanoparticles have decreased cell viability from 99% to 23% with increasing concentration from 10 to100 μg/mL.

***Keywords*:** Green synthesis, Zinc Oxide nanoparticles, *Nyctanthes arbor-tristisis*, XRD, FTIR, UV-Visible, SEM, EDX and anticancer activity.

1. Introduction

Nanotechnology is one of the most important research and promising new technologies of the 21st century. A number of physical, chemical, biological and hybrid methods are employed to synthesize different types of nanoparticles **[1]**. The physical and chemical methods are mostly used to synthesize nanoparticles. The presence of toxic compounds in these methods limits their applications and also it costs high. Therefore to avoid the toxicity, the green methods are involved for synthesize the nanomaterials using plant materials **[2-4]**. The plant based materials seems to be the best candidates for large-scale biosynthesis of nanoparticles. The advantage of using green methods is safe, cost effective and eco-friendly when compared to conventional methods **[5, 6]**. Therefore the use of non toxic leaf extracts as reducing and stabilizing agent is also an additional advantage for the green method. The presence of biomolecules in plant leaf extract, they can act as capping and stabilizing agent during synthesis process **[7]**.

The Zinc is a mineral which is very essential for human health and the zinc oxide nanoparticle has good biocompatibility to human cells. Among metal oxides, a zinc oxide nanoparticle (ZnO NPs) is a bio-safe material and it has wide range of applications in medical, industrial, agricultural, and environmental fields **[8, 9]**. The ZnO nanoparticle has more significant in controlling the growth of bacteria.

Cancer is characterized by invasive and uncontrolled cell division and the spread of abnormal cells **[10]**. Every year thousands of people worldwide die from different types of cancer. Chemotherapy and radiotherapy are most common treatments of cancer but they evoke many serious side effects **[11]**. The cytotoxic agents or cancer drugs are very few and are not very efficient **[12]**. Alternative treatment options are very less. Medicinal plants have always been used as natural remedy for many ailments and they have been a boon for many diseases and disorders. Nanoparticles synthesizing using medicinal plants extracts further enhances their efficacy as natural drugs many folds **[13-15]**. In the present work, we report for the first time synthesis of ZnO NPs from leaves of *Nyctanthes arbortristis*.

Nyctanthes arbortristis is commonly known as night jasmine or Harshringar which is the most important medicinal plant mainly used in Ayurveda **[16- 18]**. The leaf extract from the plant is used for various treatments of diseases such as sciatica, arthritis, fever, asthma, diabetes, cancer. The

phytoconstituents of plant leaf contains flavanoid, glycoside, oleanic acid, essential oils, tannic acid, carotene, friedeline, lupeol, glucose, benzoic acid have been reported for significant hair tonic, hepatoprotective, anti-leishmaniasis, anti-viral, antifungal, anti-pyretic, anti-histaminic, anti-malarial, anti-bacterial, anti-inflammatory and anti-oxidant activities **[19,20]**. The biologically synthesize ZnO nanoparticle is investigated by various characterization techniques such as X-Ray diffraction, Fourier Transform-Infrared Spectroscopy (FTIR), UV-Visible spectroscopy, Scanning Electron Microscopy and Energy Dispersive X-Ray Analysis. The cytotoxic effect is evaluated by MTT assay against colon cancer cell line (HT-29 cells).

2. Materials and methods

The Zinc Oxide (ZnO) nanoparticle was synthesized by Co- precipitation method using fresh leaf extract *Nyctanthes arbor-tristisis.* The leaves were collected around the areas of Dharmapuri. The fresh leaf extract of *Nyctanthes arbor-tristisis* was boiled for 60 min until the color of the aqueous solution changes from watery to light yellow. Then the extract was cooled to room temperature and filtered using Whatman filter paper No. 1. The 10 ml of leaf extract is mixed with 0.35M of Zinc acetate dihydrate (50ml) aqueous solution which was prepared using deionized water and allowed to stir for 2 Hrs. While stirring 2M of NaOH (50ml) was added inorder to maintain pH at 13 and was stirred for 4 Hrs. After complete stirring, the white precipitate was formed. Next, the white precipitate was rinsed repeatedly for three to four times using distilled water inorder to eliminate impurities. The precipitate was dried in an oven for 100˚C for 3 Hrs and the obtained particles were calcinated under a muffle furnace at 400˚C for 3 Hrs **[21, 22]**. Then the particle was grinded using mortar to get fine nanoparticle. The process synthesis method is shown in **Figure 1**.

3. Characterization Techniques

The crystalline nature of ZnO nanoparticle is studied using X-Ray diffraction (Shimadzu XRD 6000 X-Ray diffractometer)**.** The capping molecules and the functional groups are tested in Fourier Transform Infrared Spectroscopy (FTIR) using PerkinElmer. The optical properties of ZnO nanoparticle is characterized by a PerkinElmer Lambda 35 and the spectrum is recorded the wavelengths from 200 to 800 nm. The high resolution imaging and morphological structure of ZnO nanoparticles are determined by Scanning Electron Microscopy using the instrument Jeol JSM 6390. The Energy Dispersive X-Ray analysis is used to analyze elemental composition and stoichiometric mass percentage of ZnO nanoparticles (EV018, Carl Zeiss).

3.1. Anticancer activity

The ZnO nanoparticle is tested for anticancer using HT-29 cells by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Briefly, the cultured HT-29 cells are harvested by trypsinization, pooled in a 15 ml tube. Then, the cells are plated at a density of 1×10^5 cells/ml cells/well (200 µL) into the 96-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24-48 Hrs at 37°C. The wells are washed with sterile PBS and treated with various concentrations of the ZnO nanoparticle in a serum free DMEM medium. Each sample is replicated three times and the cells are incubated at 37°C in a humidified 5% CO_2 incubator for 24 Hrs. After the incubation period, MTT (20 µL of 5 mg/ml) is added into each well and the cells incubated for another 2-4 Hrs until purple precipitates are clearly visible under an inverted microscope. Finally, the medium together with MTT (220 µL) are aspirated off the wells and washed with 1X PBS (200 µl). Furthermore, to dissolve formazan crystals, DMSO (100 µL) is added and the plate is shaken for 5 min. The absorbance of each well is measured at 570 nm using a micro plate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC_{50} value is calculated using GraphPad Prism 6.0 software (USA) **[23]**.

4. Result and Discussion

4.1.X-Ray diffraction

The XRD is used to determine the particle size or the grain size of the zinc oxide nanoparticle. The ZnO nanoparticles are found to be hexagonal and matched with JCPDS card no.79-2205 **[24]**. From the **Figure 2**, the typical diffractions of (100), (002), (101), (102), (110), (103), (200) and (112) are attributed to ZnO nanoparticle and the maximum detection of (101) at the angle 36˚ is determined **[25]**.The particle size of the ZnO nanoparticle is calculated using Scherrer's equation.

$$D = \frac{k\lambda}{\beta \cos\theta} \text{ nm}$$

Where k = Scherrer's constant (0.9), λ = Wavelength of X-ray (1.54×10^{-10} m), β = Full Width at Half Maximum (FWHM), θ = Bragg's angle.

From the **Table 1**, it shows that the values of particle size for the ZnO nanoparticles are calculated from the above equation using three strongest peaks. Therefore the average particle size is 25 nm for the ZnO nanoparticle using *Nyctanthes arbor-tristis* leaf extract.

The particle size of the ZnO nanoparticle indicates more crystalline in nature which also confirmed theoretically using Scherrer's equation.

4.2.Fourier Transform Infrared Spectral Analysis (FTIR)

The **Figure 3** shows the FTIR spectrum of zinc oxide nanoparticles using *Nyctanthes arbor-tristis* leaf extract and ranges from 4000 cm^{-1} - 400 cm^{-1}. The FTIR spectrum is used to investigate the functional groups presents in ZnO nanoparticles with capping agent of leaf extract *Nyctanthes arbor-tristis*. The biomolecules present in the leaf extract which is responsible of reducing and capping agent for the ZnO nanoparticle. The FTIR spectrum shows the strongest peak at 3441.89 cm^{-1} indicates hydroxyl functional groups in alcohol and phenolic compounds. The peak at 490.48 cm^{-1} confirms the presence of ZnO nanoparticles. The peak at 2924.65 cm^{-1} is due to the aromatic C-H stretching vibration. The peak around at 1632.59 cm^{-1} is assigned to carbonyl and carboxylic (C=O) stretching bands of peptide linkages. The absorption band at 1549.30 cm^{-1} is attributed to N–H bending vibration. The peaks at 1436.48 cm^{-1} and 1110.28 cm^{-1} are due to C-C and C-N stretching vibration respectively. The aromatic C-H bending vibration is observed at 841.21cm^{-1} **[26, 27].** The presence of *Nyctanthes arbor-tristis* leaf extract is considered as the capping ligands which gives the stability to the ZnO nanoparticles.

4.3.UV-Visible spectroscopy

The UV-Visible spectroscopy is used to determine the optical properties and has wide range of applications in biomedical science **[28]**. The zinc oxide nanoparticles have attracted great attention towards the optical properties. The UV absorption spectrum of ZnO nanoparticle shows the strongest absorption peak at 391.31 nm and the binding gap energy is 3.18 eV. The color of the ZnO nanoparticles is white in color due to its surface plasmon resonance which confirms that the solution is free from impurities. The **Figure 4** shows the absorption peak of ZnO nanoparticle using *Nyctanthes arbor-tristis* leaf extract.

4.4.Scanning Electron Microscopy

The scanning electron microscopy is used to determine the surface morphology and shape of the ZnO nanoparticles. The micrographs of ZnO nanoparticles using fresh leaf extract *Nyctanthes arbor-tristis* are shown in **Figure 5**. The precursor is used as zinc acetate then the zinc oxide

molecules are grown slowly, forms spherical shape and also agglomeration in the nanoparticles. The SEM results that the ZnO nanoparticles are uniformly distributed and they are in spherical shape **[29]**.

4.5.Energy Dispersive X-Ray analysis (EDX)

The elemental composition of the ZnO nanoparticles is determined by EDX analysis. The **Figure 6** shows the single peak of zinc and oxygen is present between 0 and 2. The two peaks of zinc are present between 8 and 10 **[30]**. The presence of elements in the sample confirms that the nanoparticles are formed high purity without any external disturbance. The stoichiometric mass percentage of zinc and oxygen are 66% and 34% respectively and is shown in **Table 2**.

4.6. Anticancer activity of green synthesized nanoparticles

The anticancer activity of ZnO nanoparticles are performed by MTT assay. The cell viability assay is one of the most important parameter which gives more information about survival and death cells **[31]**. The cell viability of synthesized ZnO nanoparticles using fresh leaf extract *Nyctanthes arbor-tristis* against HT-29 cell lines for different concentrations is shown in **Figure 7**. The HT-29 cell lines are treated with different concentrations which ranges from 10 to100 µg/mL to assess the percentage of cell viability. The cell viability of HT-29 cell lines is decreased with increasing concentration of ZnO nanoparticles from 10 to100 µg/mL which are given in **Table 3**. The synthesized ZnO nanoparticles have decreased cell viability from 99% to 23%. The half maximal inhibitory concentration of the ZnO nanoparticles has 61% of cell viability at IC_{50}. This result clearly indicates that the ZnO nanoparticles have highly cytotoxic effect against HT-29 cell lines **[32]**. The microscopic images of green synthesis of ZnO nanoparticles using fresh leaf extract *Nyctanthes arbor-tristis* against HT-29 cell lines is shown in **Figure 8**.

5. Conclusion

The simple and economical green methods are used to synthesize ZnO nanoparticles using fresh leaf extract of *Nyctanthes arbor-tristis*. The ZnO nanoparticles are characterized by XRD, FTIR, UV-Visible, SEM and EDX. The X-Ray diffraction proved that the ZnO nanoparticles are crystalline in nature and particle size is about 25 nm. The peak at 490.48 cm^{-1} confirms the presence of ZnO nanoparticles. The absorption peak at 391.31 nm clearly indicates the synthesis of ZnO nanoparticles along with formation of white color which indicates that the particle is free from

impurities. The surface of the ZnO nanoparticles is spherical shape which is analyzed by SEM. The stoichiometric mass percentage of zinc and oxygen is 66% and 34% respectively which is determined by EDX spectrum. The ZnO nanoparticles show the cell viability of HT-29 cell lines decreased with increase concentration. Hence the green synthesized ZnO nanoparticles have great advantage of less toxicity, low cost and good biocompatibility.

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Figure1: The systematic representation of preparing ZnO nanoparticles

Figure 2: The XRD analysis of ZnO nanoparticle using leaf extract *Nyctanthes arbor-tristis*

Figure 3: The FTIR spectrum of ZnO nanoparticle using *Nyctanthes arbor-tristis* leaf extract

Figure 4: The UV-Visible spectroscopy of ZnO nanoparticle using *Nyctanthes arbor-tristis* leaf extract

Figure 5: The SEM micrographs of ZnO nanoparticles using *Nyctanthes arbor-tristis* leaf extract.

Figure 6: The EDX spectrum of ZnO nanoparticles using aqueous leaf extract *Nyctanthes arbor-tristis*.

Figure 7: The cell viability of synthesized ZnO nanoparticles using fresh leaf extract *Nyctanthes arbor-tristis* against HT-29 cell lines for different concentrations

Figure 8: The microscopic images of green synthesis of ZnO nanoparticles using fresh leaf extract *Nyctanthes arbor-tristis* against HT-29 cell lines.

Table 1: The particle size of ZnO nanoparticle for the three strongest peaks.

Table 2: The stoichiometric mass percentage of Zinc and oxygen using fresh leaf extract *Nyctanthes arbor-tristis*

Table 3: Cell viability of ZnO nanoparticles

Figures

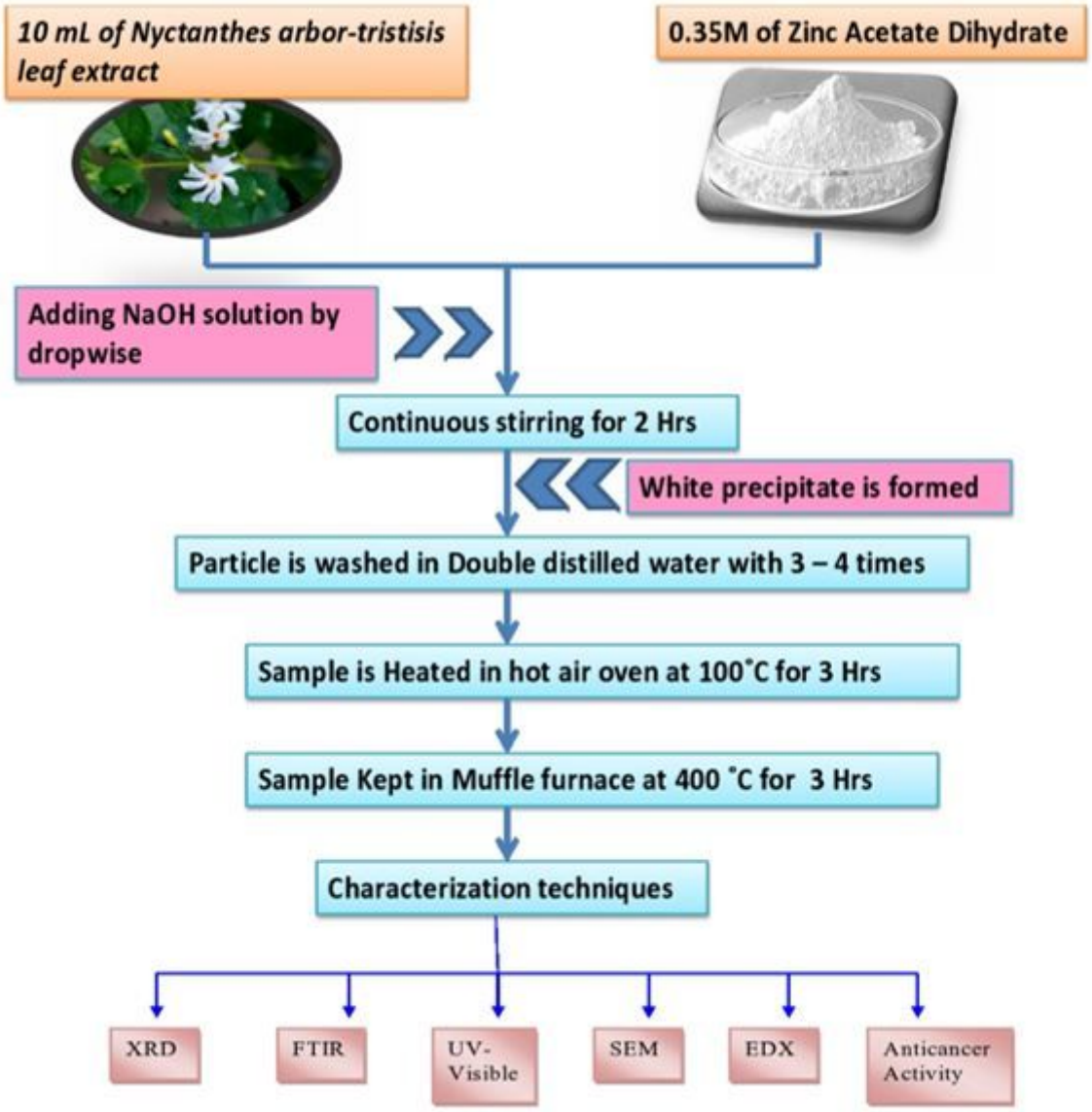

Figure 1

The systematic representation of preparing ZnO nanoparticles

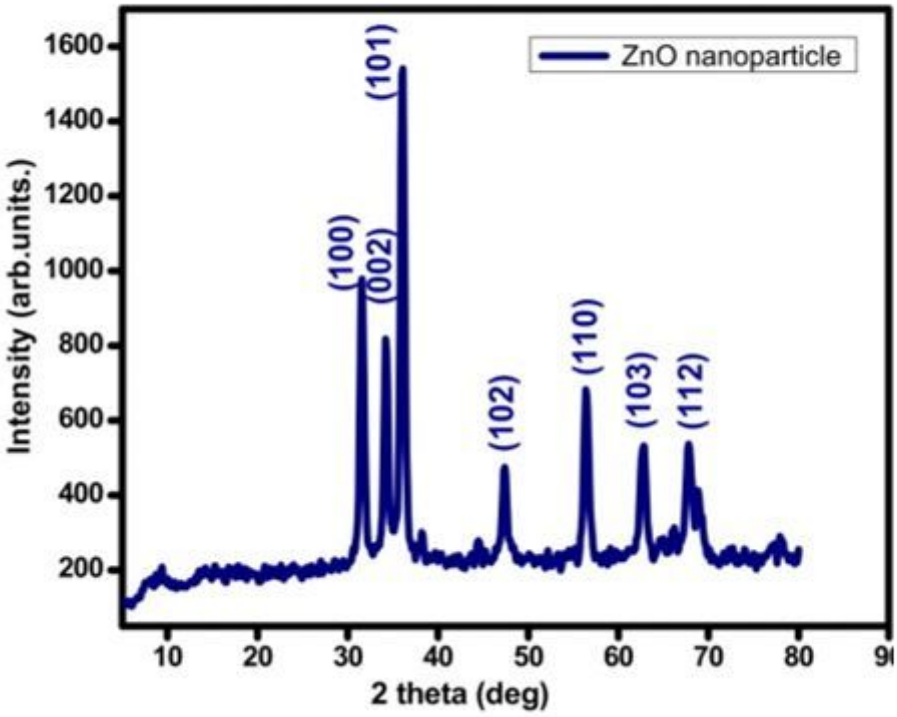

Figure 2

The XRD analysis of ZnO nanoparticle using leaf extract Nyctanthes arbor-tristis

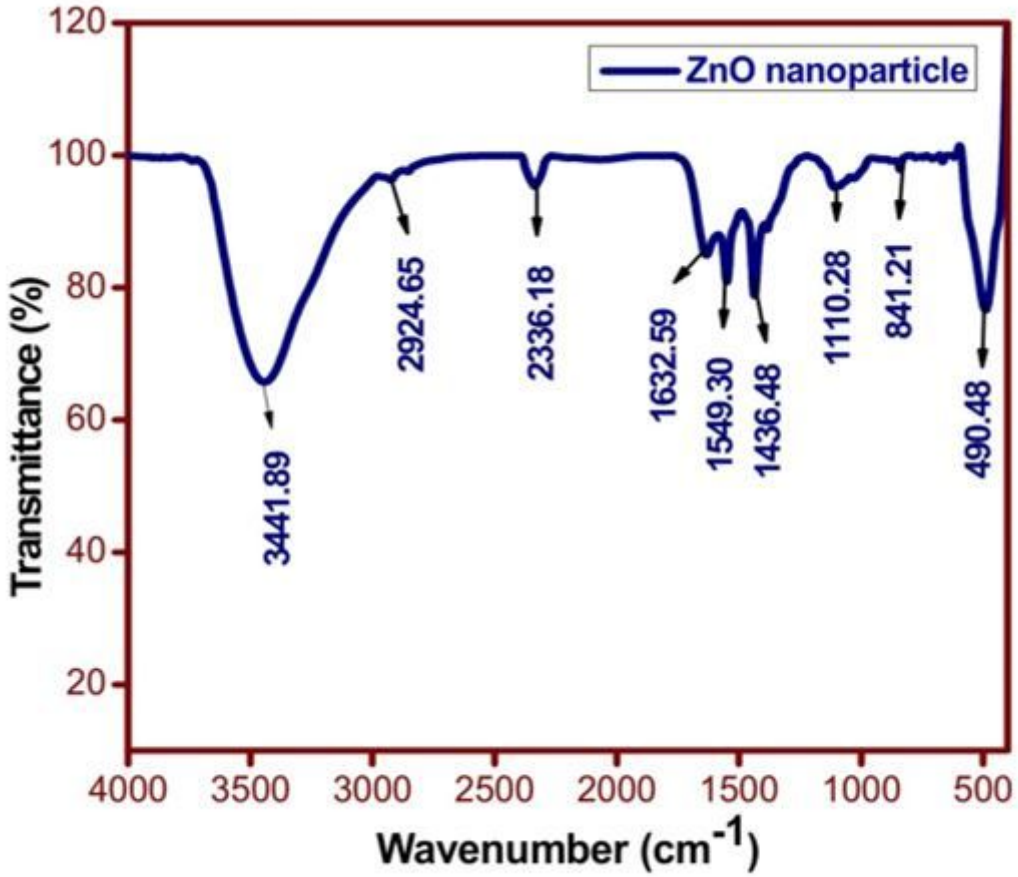

Figure 3

The FTIR spectrum of ZnO nanoparticle using Nyctanthes arbor-tristis leaf extract

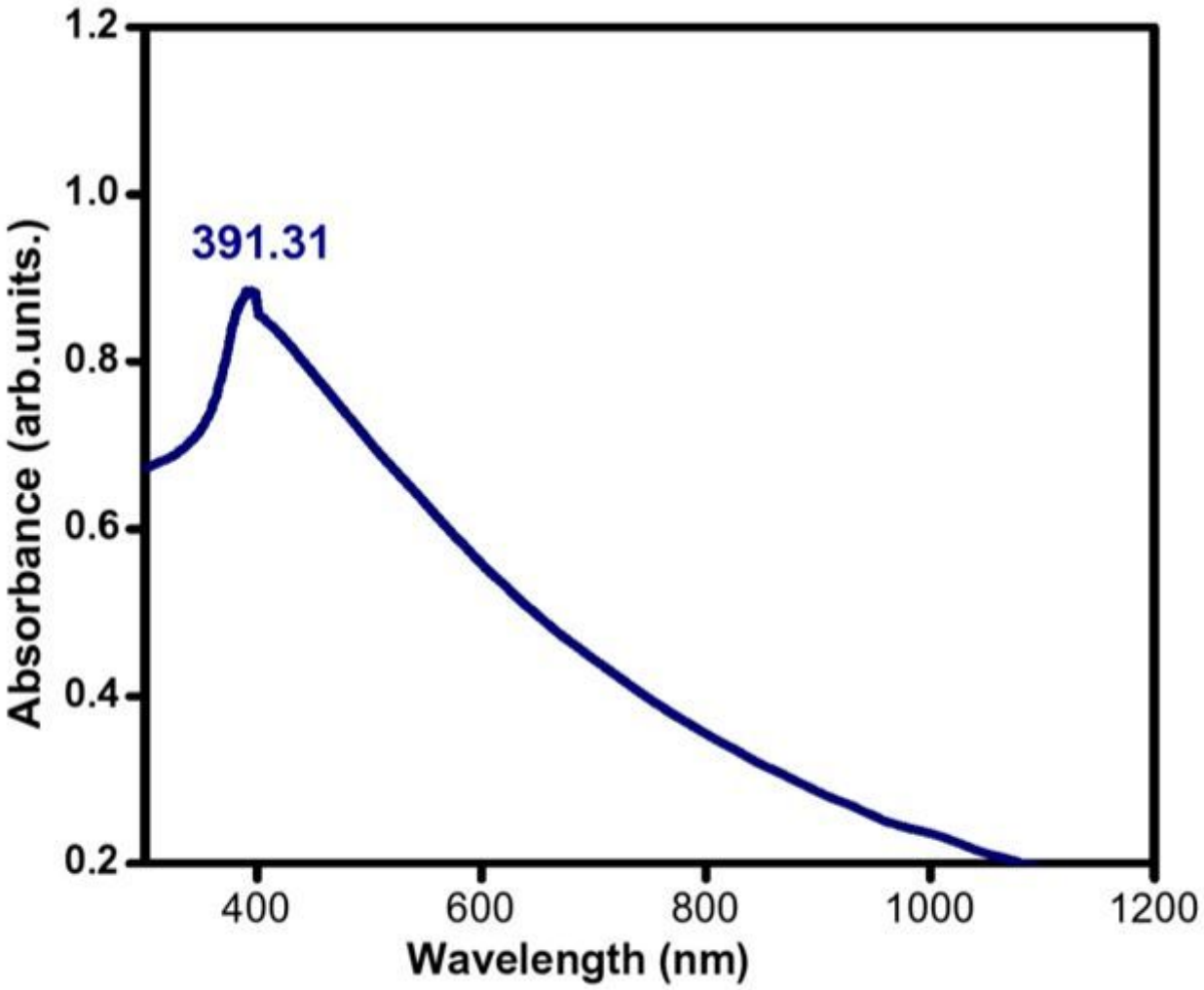

Figure 4

The UV-Visible spectroscopy of ZnO nanoparticle using Nyctanthes arbor-tristis leaf extract

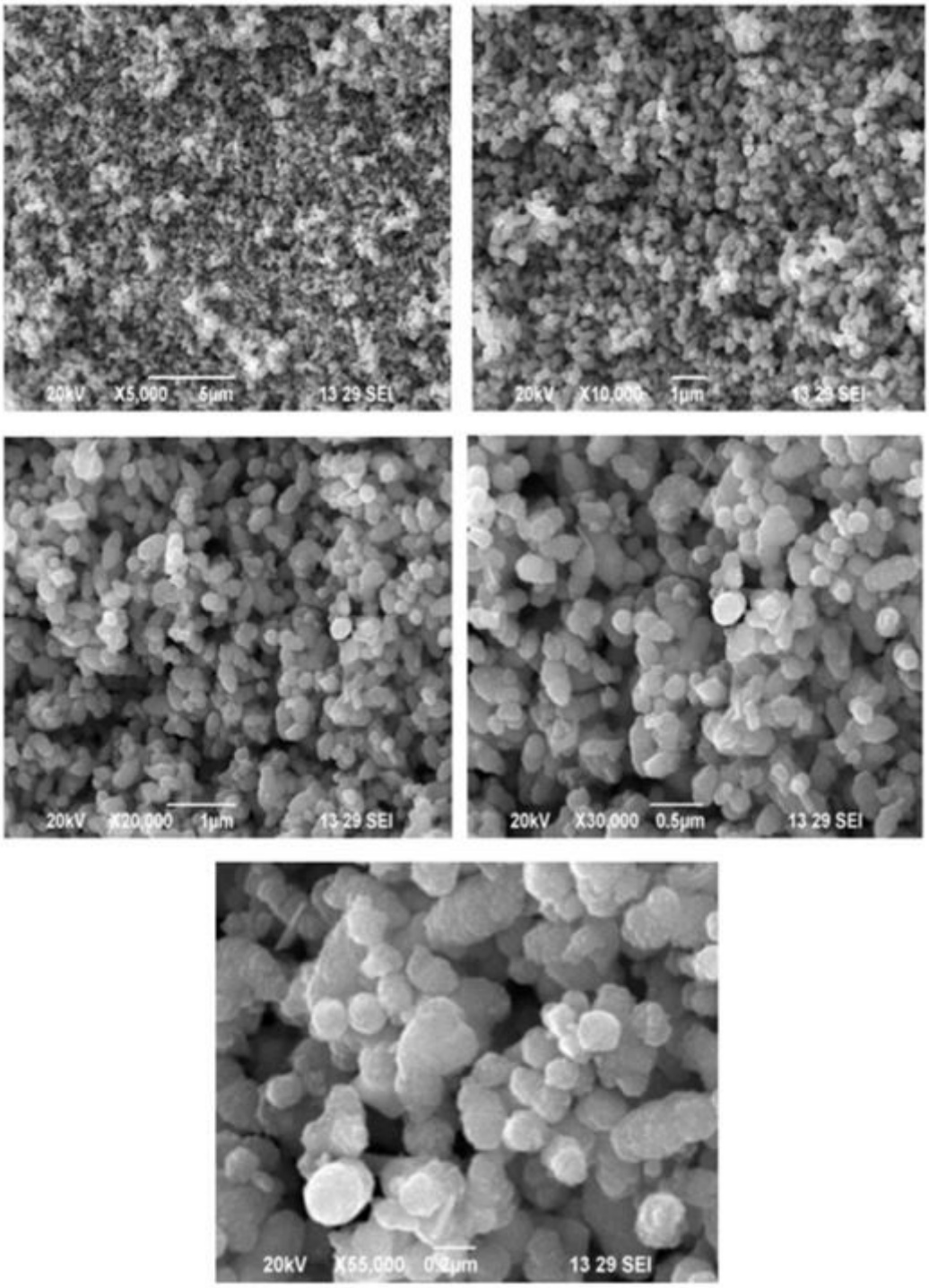

Figure 5

The SEM micrographs of ZnO nanoparticles using Nyctanthes arbor-tristis leaf extract.

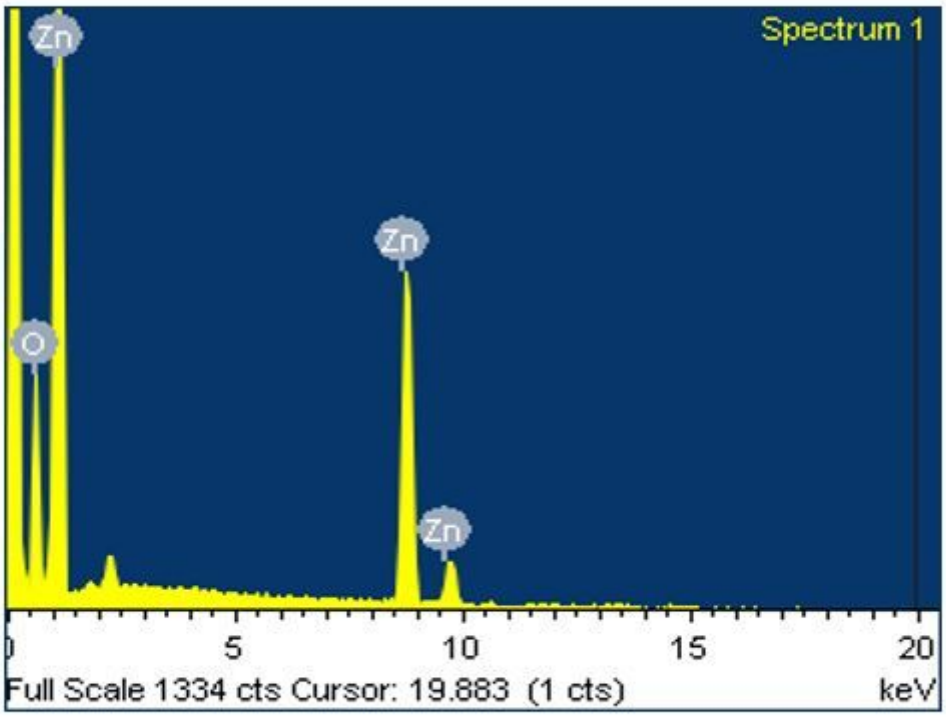

Figure 6

The EDX spectrum of ZnO nanoparticles using aqueous leaf extract Nyctanthes arbor-tristis.

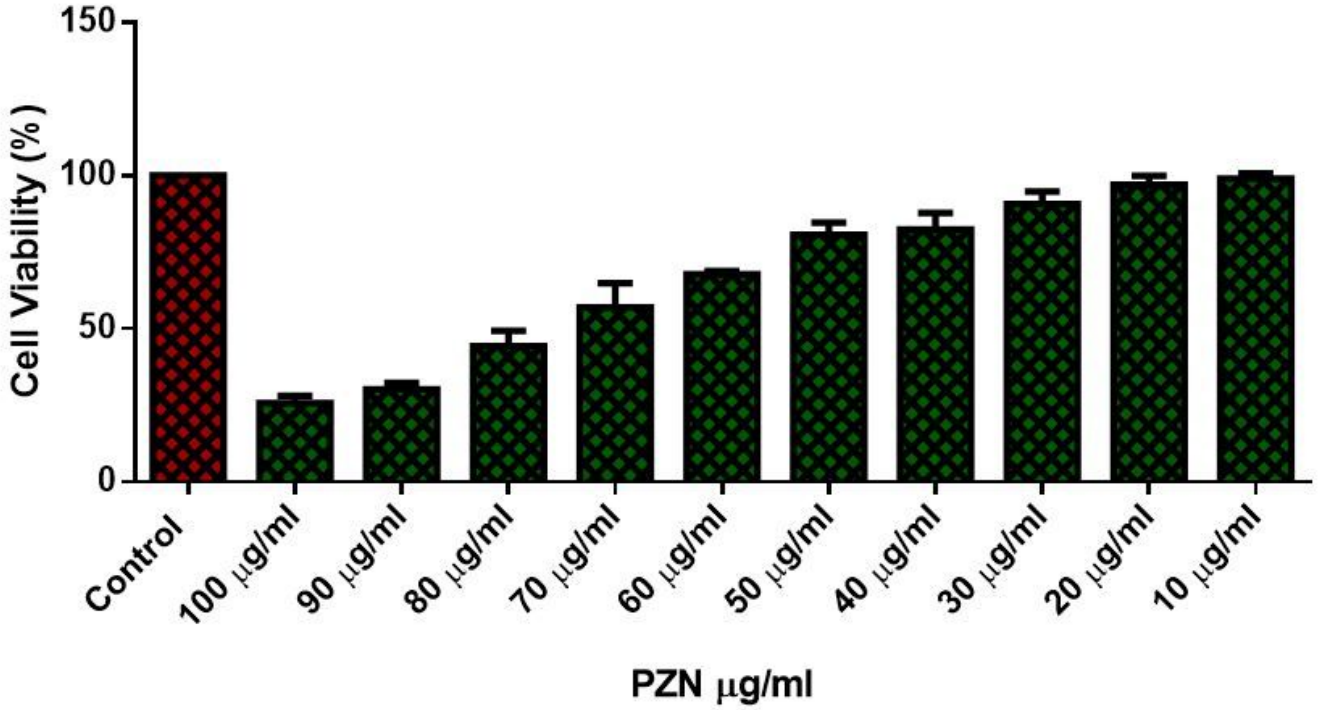

Figure 7

The cell viability of synthesized ZnO nanoparticles using fresh leaf extract Nyctanthes arbor-tristis against HT-29 cell lines for different concentrations

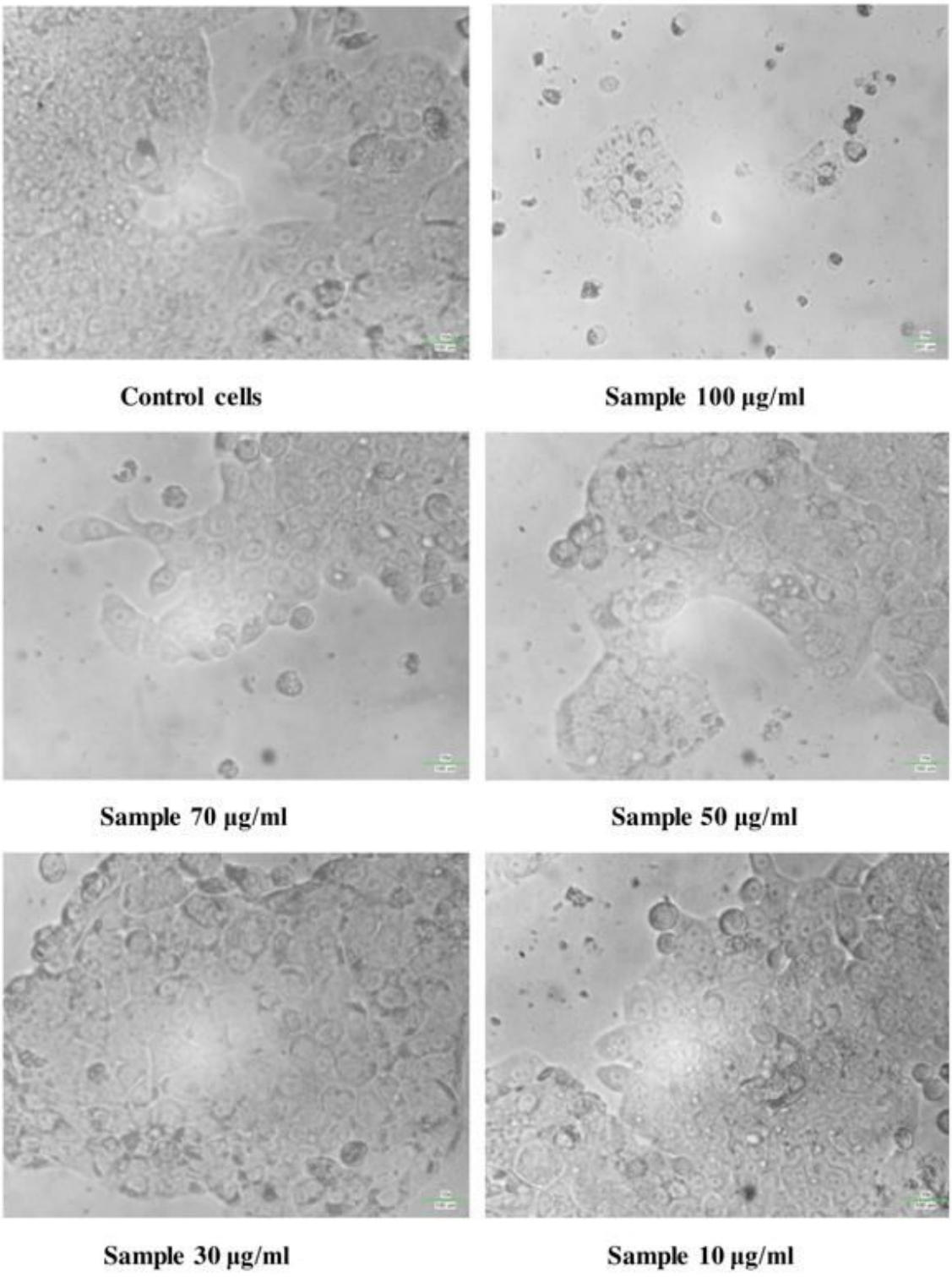

Figure 8

The microscopic images of green synthesis of ZnO nanoparticles using fresh leaf extract Nyctanthes arbor-tristis against HT-29 cell lines.