ECC
Eurasian Chemical Communications
Original Research Article
http://echemcom.com

Optimization of biosynthesized Zn nanoparticles by poisonous *Taxus baccata* leaves extract and evaluation of their effect on the bacterias and MCF-7 cancer cells

Sona Sarli[a], Nahid Ghasemi[a,*]

[a]*Department of Chemistry, Arak Branch, Islamic Azad University, Arak, Iran*

Received: 14 September 2019, Accepted: 16 November 2019, Published: 02 December 2019

Abstract

Recently, phyto nanotechnology has proposed new methods for the biosynthesis of nanoparticles and is also an eco-friendly, stable, rapid, simple, and cost-effective method. The present study reported the green synthesis of Zn nanoparticles (Zn NPs) by *Taxus baccata* extract, and their performance was tested against three types of bacteria and a type of cancer cells. These experiments are designed in a fully compatible environment. In order to evaluate effective parameters, Ultraviolet-visible (UV–Vis) spectroscopy was used. In addition, we had a comparative study of the performance of synthesized nanoparticles on bacteria, and a comparative study on the pure extract of this plant as an anticancer agent along with biosynthesized Zn nanoparticles. The SEM and FESEM results revealed hexagonal structure with the particle size of 20 nm. TEM analysis of the synthesized nanoparticles showed hexagonal particles with an average size of 20-25 nm. FTIR analysis confirmed the reduction of functional groups, alkaloids, corresponding of synthesis Zn nanoparticles from their salt solution. The antibacterial activity of *Taxus baccata* extract, Zn nanoparticles, and zinc nitrate solution was tested against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. Anticancer activity of *Taxus baccata* extract, zinc nitrate solution and synthesized Zn nanoparticles on cancer cells was assessed by Breast cancer cell line MCF-7. The results showed that the synthesized Zn nanoparticles using plant leaf extract had strong anti-cancer activity on MCF-7 cells. On the other hand, the synthesized metal nanoparticles had no effect on the three types of bacteria.

Keywords: *Taxus baccata*; FESEM analysis; TEM analysis; Zn nanoparticles; antibacterial; anticancer.

Introduction

Today, nanotechnology is one of the most important technologies which rely on nanoparticles synthesis and modulation, requiring considerable modification of metals properties [1]. Recently, phyto nanotechnology has proposed new methods for the synthesis of nanoparticles and is also an eco-friendly, stable, rapid, simple, and cost-

*Corresponding author: Nahid Ghasemi
Tel: +98 (86)34135421, Fax: +98 (86)34135421
E-mail: anahid3@gmail.com

effective method. Phyto nanotechnology has many advantages such as scalability, biocompatibility, and medical applicability of synthesizing nanoparticles using water, and the universal solvent, as a reducing medium [2]. Thus, producing plant-derived nanoparticles by available herbal materials and the non-toxic nature of plants are suitable for accomplishing the wide demand of nanoparticles with practical applications in environmental and biomedical areas. Different parts of plants (including leaves, fruits, stems, roots, and their extracts) have capability for metal nanoparticles' synthesis [3]. Mechanism and components which are responsible for plant-based synthesis of nanoparticles have not yet been interpreted well. Research results have confirmed that proteins, organic acid, vitamins, amino acids, as well as secondary metabolites, such as flavonoids, terpenoids, alkaloids, polyphenols, and polysaccharides can act as capping and stabilizing agents in preparing nanoparticles and play significant role in the reduction of metallic salts [4].

Interesting properties of zinc including wide direct band gap of 3.3 eV at room temperature and high excitant binding energy of 60 meV have made it an attractive metal for researchers [5]. Zinc, as an unbeatable substance, is applied in transparent electronics, chemical sensors, ultraviolet (UV) light emitters, piezoelectric devices, personal care products, spin electronics, coating and paints. Semiconducting, pyro electric, and piezoelectric properties are also ascribed to zinc [6]. It is suggested to use plants and plant extracts as more echo-compatible and controllable methods instead of chemical and physical ones for metal nanoparticles biosynthesis [7]. Enzymes [8], plant leaf extracts [9] and bacteria [10] have an important role in producing Zn nanoparticles (Zn NPs) by green synthesis route.

Different types of methods are used for metal nanoparticles synthesis because of their various applications. The usual chemical methods have certain limitations during the synthesis procedure or in later applications. Different chemical reducing agents are used during the process of metal particles' chemical reduction, however; they are mostly toxic and harmful to the environment. The physical methods need the use of heavy devices, high and varied pressure and temperature [11], large space area, while an eco-friendly and affordable method is suitable for this purpose.

One of the wide applications of Zn is in cosmetics (like sunscreen lotions) because it can act as a filter against UV lights [12]. Zn has a widespread use in biomedical applications like drug delivery, anti-cancer, antibacterial, antifungal, anti-diabetic and agricultural properties [13,14]. It is also utilized very usefully in different industries such as rubber manufacturing, sulfur and arsenic removal from wastewater, protein adsorption, dye and dental applications. In addition to pyro electric and piezoelectric properties [15], Zn NPs are used to eradicate aquatic weed which is resistant to all types of physical, chemical and mechanical means of eradication techniques [16].

The medicinal value of *T.baccata* is due to the presence of Paclitaxel, with the brand name of Taxol, in its needle leaves. *Taxol* has an abnormal cell division, which stops the DNA transcription in the G2 / M step of mitotic division, and leads to the death of cells that are being proliferated. In 1977, FDA recognized Taxol for uterine cancer and breast cancer treatment. Although there are several new methods such as cell

culture which produce this valuable medicine, plant extract methods are still of great importance. The leaves of *Taxus* species are utilized much easier than other parts. Research has revealed that the raw extract of twigs and leaves have an inhibitory role against the growth of many human carcinoma engrafts after oral injection to mice [17]. Strong cytotoxicity of some other toxoids (like cephalomannine) to cancer cells has been reported, in addition to paclitaxel [18-22]. So, the synergistic properties of taxoid compounds in *Taxus* might be responsible for the antitumor effect of *Taxus* extract. Recently, oral administration of ZnO nanoparticles synthesized using *Taxus* extract has been resulted in the pharmacokinetic profiles of the main taxoids. Until now, different analytical methods have been studied and used for measuring the main taxoid compounds in the extracts of *Taxus* leaves. High-performance liquid chromatography (HPLC) with UV detection, high-speed countercurrent chromatography (HSCCC) and enzyme-linked immunosorbent assays (ELISA) are the mostly utilized methods of taxoids determination in herbal extracts [23-26]. Recently, a few LC–MS methods have determined paclitaxel and other taxoids in the extract of three *Taxus* species [27-30]. However, the absence of main taxoids in plasma has been reported after oral administration of the extract of *Taxus*. Plants, bacteria, algae, and fungi are considered as suitable options for biosynthesis of metal nanoparticles since they are very cost-effective, safe and eco-friendly. That is why, in recent years, stable and available plants resources for biocompatible nanoparticles synthesis have attracted the attention of many researchers.

Zinc is also widely used for cancer researches. It is a metal with an oxidation state of +2, with five stable isotopes. Zinc is considered as a very important and abundant element in the body after iron. The whole amount of Zinc in the body is estimated around 2g. The human body requires 15 mg of Zinc per day [31-37]. However, it can be found in all body tissues and fluids. Zinc affects humoral and cellular immunity and plays a major role in the immune system. It helps the entirety of cell and organ maintenance, by stabilizing the cellular and molecular components of the membrane. Zinc controls a large number of enzymes in the human body, which participates in synthesis and degradation of carbohydrates, proteins, lipids, nucleic acid and in the metabolism of other micronutrients [38].

Extract of *Taxus baccata* (Figure 1) as a source of natural alkaloids (namely *Taxanes* or *Taxoids*) was employed for Zn NPs synthesis as a reducing agent that is shown in Figure 2. Several experiments were carried out to investigate the influence of pH adjustment, the volume of extract, zinc nitrate solution, temperature and time on Zn NPs formation and their optimal condition and characterized by UV-Vis in each condition. In addition, the synthesized Zn NPs were characterized using SEM, FESEM, TEM and FT-IR.

Anti-bacterial effects of *Taxus baccata* extract, zinc nitrate solution and Zn nanoparticle synthesized were investigated against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. Anti-cancer activity of the extract, zinc nitrate solution and Zn nanoparticles was tested by breast cancer cell line MCF-7. Anticancer activity of the *Taxus baccata* extract containing Taxol was also studied and the results were compared with those of anticancer effects of Zn nanoparticles and zinc nitrate solution. In this study, the green synthesis of Zn nanoparticles (Zn NPs) by *Taxus baccata* extract was

characterized by UV-Vis, SEM, FESEM, TEM and FT-IR analysis; their performance was tested against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. Anticancer activity of *Taxus baccata* extract, zinc nitrate solution and synthesized Zn nanoparticles on cancer cells was assessed by breast cancer cell line MCF-7.

Materials and methods

Material preparation

The leaves, trunks, and shells of *Taxus baccata* prepared from the mountains of Gorgan in Iran, are shown in Figure 1. Zinc nitrate ($Zn(NO_3)_2$) was purchased from Sigma-

a) b)

Figure 1. a) *Taxus baccata* plant b) leaf and fruit

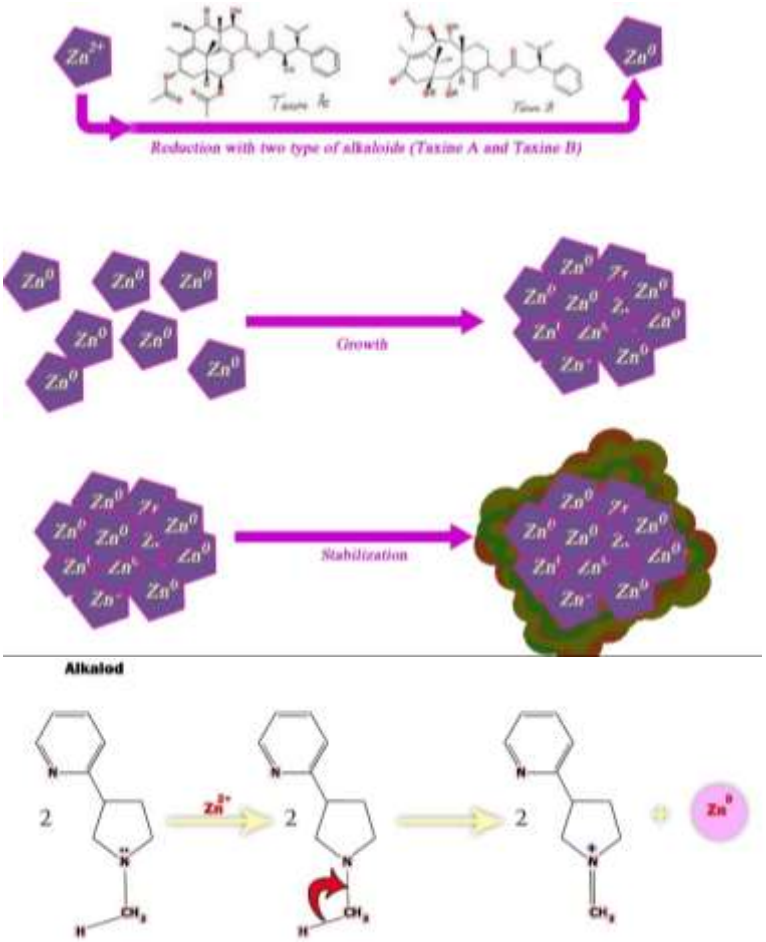


Figure 2. A schematic representation of zinc nanoparticle synthesis using Taxus baccata extract

Aldrich, and the other chemicals such as hydrochloric acid, and sodium hydroxide based on analytical grade were purchased from Merck: Darmstadt, Germany. Also, deionized water was used for all experiments. Staphylococcus aureus (ATCC25923), Escherichia coli (ATCC 1399) and Pseudomonas aeruginosa (ATCC 1430) were prepared from Iranian research organization for science and technology. Breast cancer cell line MCF-7 was purchased from Pasteur Institute - Cell Bank.

Preparation leaf extracts of Taxus-baccata

Leaves were collected from the *Taxus baccata* tree leaves, were dried and powdered after being washed thoroughly with double distilled water.

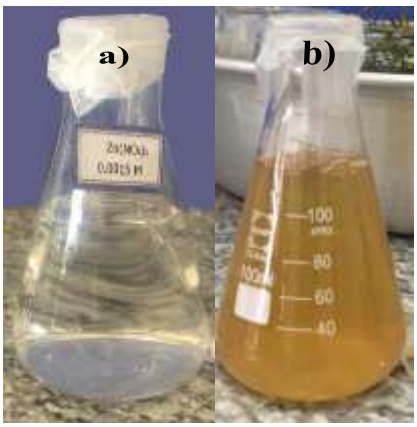


Figure 3. a) $Zn(NO_3)_2$ solution, b)*Taxus baccata* extract

20 g of leaves powder was heated in the 100 mL double distilled water and heated at 40 ºC for 24 h. The heated materials were filtered through Whatman No.1 filter paper, centrifuged at 5000 rpm for 5 min, and a clear solution of the extracts was stored at 4 ºC for further experiments.

Synthesis of Zn nanoparticles

In order to find the best synthesis condition of Zn nanoparticles by *Taxus baccata* extract, the influence of pH, volume of extract, salt solution concentration, temperature and time were studied. When the color changing occurred in the reaction mixture, nanoparticles formation and aqueous metal ion reduction using plant extract were observed (Figure 3). For this purpose, UV-Vis spectrophotometry was used and each sample was examined in the range of 250-400 nm. Metal-based nanoparticles have many free electrons that move by conduction and balance bands which cause surface plasmon resonance (SPR) in collision with the UV light. This spectrum records vibrations of the free electrons of nanoparticles. In this work, a high peak which was appeared in the visible area also confirmed the formation of metal nanoparticles.

Effect of pH on Zn nanoparticle synthesis

0.5 mL extract and 0.001 M zinc nitrate solution were added to 7 vials. The pH of each vial was adjusted to 2, 4, 5, 6, 7, 8, and 9 by using 0.1 M NaOH and 0.1 HCl and the sample was stirred at the stirring rate of 150 rpm for 30 min at room temperature. By adding metallic salt solution, the color of solutions obviously changed from light yellow to dark yellow, which indicated the formation of Zn nanoparticles. After a while, the solutions were centrifuged at 5000 rpm for 30 min, separated from the sediment. Finally, UV spectrum was taken and the best pH for the synthesis was determined

and adjusted for next experiments (Figure 4-a).

Effect of extract volume on Zn nanoparticle synthesis

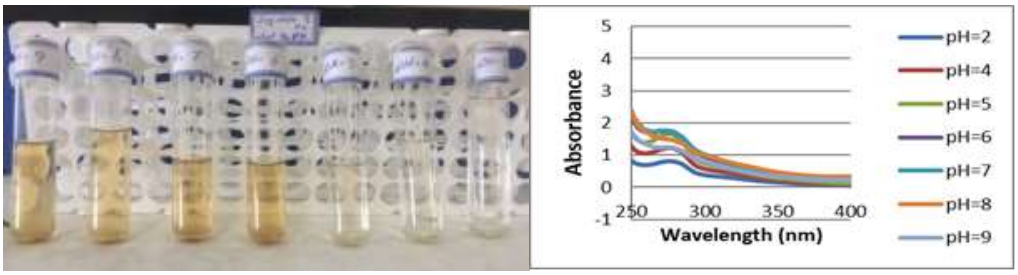
5 mL of 0.001 M zinc nitrate was poured into 7 vials and 0.1, 0.2, 0.25, 0.5, 1, 1.5 and 2 mL of *Taxus baccata* extract solution were added to them. Then, pH of all vials was adjusted to the optimized pH and the solutions were stirred at a stirring rate of 150 rpm for 30 min at room temperature. After that, the solutions were centrifuged and separated from the sediment and UV spectra were recorded as the previous step. Thus, the best volume was determined and used for further experiments (Figure 4-b).

Effect of zinc nitrate concentration on Zn nanoparticle synthesis

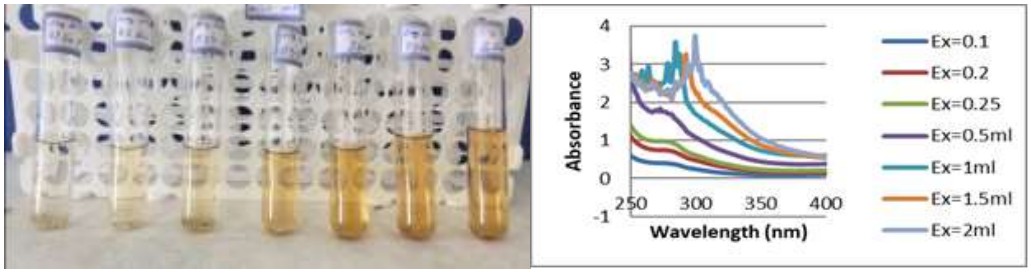
In order to optimize the concentration of metallic salt solution, 2 mL of *Taxus baccata* extract was poured into 6 vials, then 5 mL of 0.0005 M, 0.001 M, 0.0015 M, 0.002 M, 0.0025 M, and 0.003 M zinc nitrate were added to them. PH of all vials was adjusted to the optimized pH and after a while, the solutions were stirred at stirring rate of 150 rpm for 30 min. Then, the solutions were separated from the sediment and UV spectrum was taken. Therefore, the concentration was optimized and used for the next experiments (Figure 4c).

Effect of temperature on Zn nanoparticle synthesis

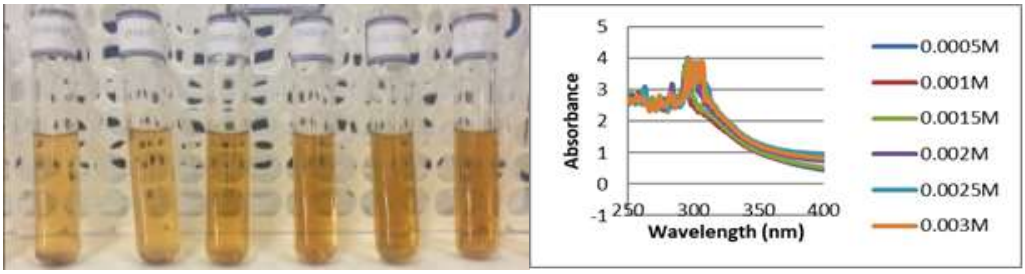
According to the previous steps, 2 mL of the leaves extract and 5 mL zinc nitrate (0.0025 M) were poured into 8 vials and pH was set at 7.



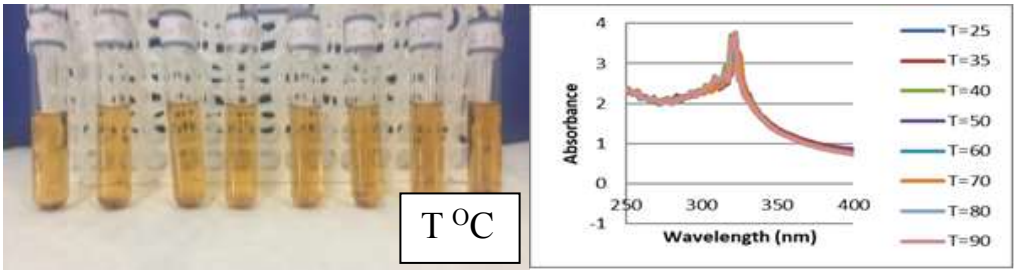
a)



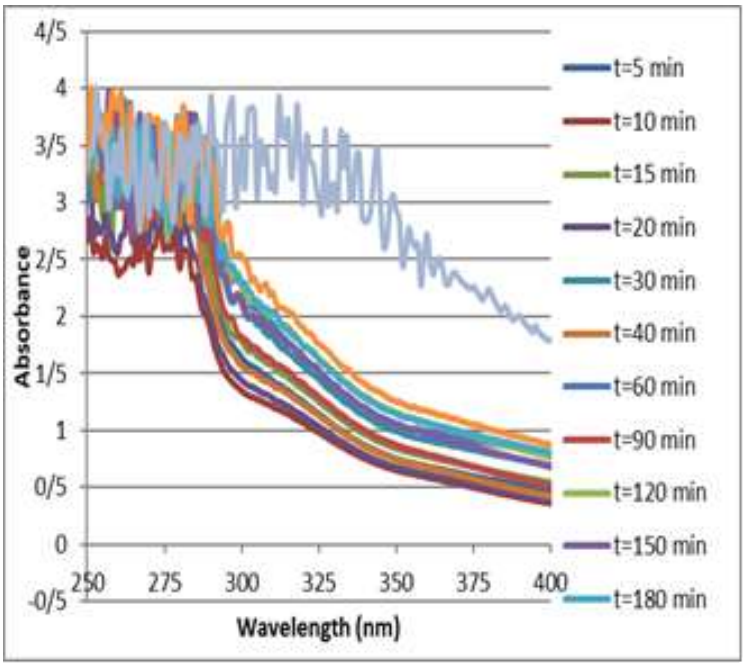
b)



c)



d)



e)

Figure 4 a-e). a) Effect of pH on Zn nanoparticles synthesis by *Taxus baccata* extract (0.5 ml of extract solution,5 ml of 0.001 M Zinc nitrate, time: 30 min, stirring rate 150 rpm), 4-) Effect of *Taxus baccata* extract solution on Zn nanoparticles synthesis (pH of the solution was 7, 5 ml of 0.001 M Zinc nitrate, time: 30 min, stirring rate 150 rpm), c) Effect of nitrate solution concentration on Zn nanoparticles synthesis by *Taxus baccata* extract (pH of the solution was 7, 2 ml of the *Taxus baccata* extract, time: 30 min, stirring rate 150 rpm), d) Effect of temperature on Zn nanoparticles synthesis by *Taxus baccata* extract (pH of the solution was 7, 2 ml of the *Taxus baccata* extract, 0.0025 M Zinc nitrate, time: 30 min, stirring rate 150 rpm), 4-e) Effect of time on Zn nanoparticles synthesis by *Taxus baccata* Extract (pH of the solution was 7, 2 ml of the *Taxus baccata* extract, 0.0025 M Zinc nitrate, temperature 25˚C, stirring rate 150 rpm)

Then, all vials were heated at 25, 35, 40, 50, 60, 70, 80, 90 ˚C for 1 h. In the next step, all samples were centrifuged and UV spectrum was recorded with the same conditions as before. So, the optimal temperature was obtained (Figure 4-d).

Effect of time on Zn nanoparticle synthesis

Determining the appropriate time for metallic nanoparticles synthesis was an important step because zinc ions were increased and released at the specific time, then the surface plasmon resonance were decreased. To this end, 2 mL of the *Taxus baccata* extract and 5 mL zinc nitrate (0.0025 M) were poured into 13 vials and the pH was set at 7. Then the solution was heated to 25 ˚C, and centrifuged. Finally, the UV spectrum was taken after 5, 10, 15, 20, 30, 40, 60, 90, 120, 150, 180, 210 and 1440 min,

respectively. According to the results, the best time for nanoparticles synthesis was determined (Figure 4-e).

Characterization of the used instruments
Characterization of the green synthesized Zn nanoparticles using Taxus baccata leaves extract
According to the previous experiments and obtained results from the UV spectra of parameters under investigation (Figure 4-a, to 4-e), the optimal parameters for Zn nanoparticles synthesis using the extract were determined. The results of UV-Vis showed that Zn nanoparticles should be synthesized again. So, 20 mL of the extract was added to 50 mL of 0.0025 M zinc nitrate solution, pH was set 7 and the sample was placed in the shaking incubator at 25 °C for 24 h. Then, it was centrifuged for 5 minutes, then washed and dried.

The obtained Zn nanoparticles were characterized by using different techniques such as SEM, FESEM, TEM and FTIR.

Determination of antibacterial activity of Zn nanoparticles
The synthesized Zn nanoparticles were examined in terms of their antimicrobial activity by agar diffusion method against different three bacterial cultures. An equal count of the initial bacterial culture with a concentration of 0.5 McFarland was subcultured on nutrient agar. Using gel puncture method, 10 Wells of 6 mm diameter were made on nutrient agar plates.

All three case study solutions including pure *T.baccata* extract, zinc nitrate, and Zn nanoparticles were diluted individually to obtain seven serial concentration titers as follows: (1/1): 50 mM, (1/2): 25 mM, (1/4): 12.5 mM, (1/8): 6.25 mM, (1/16): 3.1 mM, (1/32): 1.5 mM and (1/64): 0.75 mM. Several sets of three selected bacterial culture plates were provided for investigation of the antibacterial effects of three case study solutions. Equal volumes of 100 µl of each dilution were applied to a set of three selected bacterial culture as described above.

All plates were evaluated after a 24 h incubation period at 370 C. The inhibition zone of the bacteria was accurately measured by the caliper and the mean diameter of the bacterial inhibition zone and its comparison with positive and negative control wells were considered as indicators of antibacterial activity. Each assay was repeated for three times as described previously [43-45].

Minimum inhibitory concentration (MIC) test
The minimum concentration of growth inhibitory was determined. Like the previous steps, serial dilutions of each case study solutions were prepared. Using a 96-well plate, a set of 7 wells was considered separately for each study materials containing 100 µl of the serial diluents. 100 µl of brain-heart-infusion-broth) BHI (solution and 100 µl of active bacterial suspension 1.5×106cfu/ml were added to each well. The Same procedure was performed for all three bacterial species. For each run of the experiments, positive and negative controls were considered as well. Positive control wells included the relevant bacteria with BHI solution and negative control ones included the case study solution (extract, zinc nitrate or Zn nanoparticles) with BHI solution. It should be noted that to prepare negative control wells, 15 µl of each metal solution and 15 µl of BHI (7.3 g BHI in 100 mL of deionized water) were used. For the preparation of positive control wells, 15 µl of BHI were also used with each 15 µl of bacterium, separately. The plate was incubated for 24 h at 37 °C. The turbidity of the wells, which was due to the growth of the

bacteria, was investigated by reading their OD. The first dilution with no turbidity was recorded as the minimum deterrent concentration [46,47]. To reduce the experimental errors, all of the above experiments were repeated four times.

Assessment of anticancer activity by MTT assay

According to the previous step, 7 dilutions of case study solutions were prepared. The human breast cancer cell line MCF-7 was cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) at 37 °C with 5% CO_2. Equal numbers of the cells were passaged in 96-well plates (8.103cells/well) containing 100 µL of the medium for 24 h. 100 µL of different concentrations of the three case study solutions were dispersed in each well and incubated for 24 h at 37 °C with 5% CO_2. Fresh medium (100 µL) containing 0.5 mg/mL of MTT was replaced by the previous media in each well. The growth of the cells was quantified by the ability of the live cells to reduce the MTT Pink dye to a pale violet formazan product. The formazan product of MTT reduction was dissolved in DMSO after 4 h and the absorbance was measured using a microplate reader. The effect of Zn nanoparticles was measured at 570 nm as well.

Results and discussion

Ultraviolet-visible spectroscopy (UV-Vis)

In this study, Zn NPs were synthesized under different conditions. In each condition, it was attempted to determine the optimal condition for synthesis. We achieved similar spectra at 250-400nm using UV-Vis spectroscopy. It has been in agreement at the results of other similar studies.

Zn nanoparticles absorption in UV-Vis spectrum depends on fabrication method, particle size, etc. The change in coloration of the *Taxus baccata* leaves extract with aqueous zinc ions from yellow to dark yellow depicted the synthesis of Zn nanoparticles. In all figures (Figure 4-a, to Figure 4-e), there is a significant peak at 276 nm, indicating Zn nanoparticles formation, which is generally attributed to the related strong SPR of Zn nanoparticles [48,49]. A lot of noise was observed in all of the figures. As Malek Mohammadi and Ghasemi have shown in their studies, noise is observed because the area under the SPR-peak in the UV–Vis spectra is directly sensitive to the shape, size, and dielectric constant of the metal nanoparticles as well as the surrounding medium in aqueous suspensions [50] and causes the spectrum to be noisy. It was also observed that the volume of the extract, the concentration of the zinc nitrate solution and time were increased, and the maximum absorption occurred at about 330, indicating the formation of surface plasmon nanoparticles on Zn. The results are in good agreement with those of Sindhura [51], Revina [52], Badarinath Druvarao Kulkarni [53], Wesam Salema [54] and their colleagues. Sindhura et al. reported similar UV–Vis spectra after treatment of zinc nitrate (salt solution) (0.001M) using P. hysterophorus extract (55), and Devasenan et al. reported a main peak in 224 nm [56].

Scanning electron microscope (SEM), field emission scanning electron microscope (FESEM)

Scanning electron microscope is one of the electron microscopy techniques which can yield practical information about materials' surfaces in morphological studies. Electrons produce signals containing data about atoms surface composition, topography

and electrical conductivity by interacting with the sample atoms. In SEM, electrically non-conducting samples are covered with a thin conducting film such as metal or carbon. In this study, the morphologies of Zn nanoparticles synthesized by *Taxus baccata* extract were examined using MIRA3TESCAN-XMU FESEM analyzer with acceleration voltage of 15kV and 0.692 µm resolution. The structural and morphological studies of Zn nanoparticles were analyzed at 1µm and 200 nm magnifications as shown in Figure 5: a), b), c) and d). SEM and FESEM images showed the presence of a high density of well-dispersed Zn nanoparticles which were hexagonal with an average size of 20 nm to 27.64 nm.

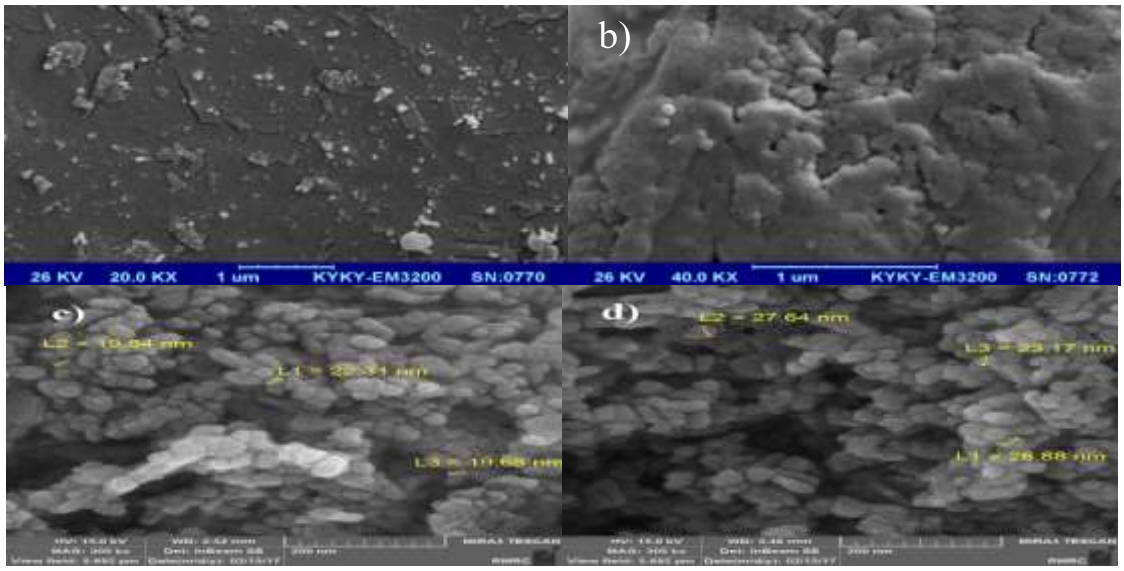


Figure 5. a, b) SEM images of Zn nanoparticles synthesized by *Taxus baccata* extract, c,d) FESEM images of Zn nanoparticles synthesized by *Taxus baccata* extract

Transmission electron microscopy (TEM)

Synthesized Zn nanoparticles by biological reduction process were prepared for TEM measurements by placing a drop over carbon-coated copper grids and waiting until the solvent was evaporated. TEM measurements were performed on Zeiss model EM900 instrument operated at 80 kV accelerating voltage. TEM images are shown in Figure 6: a) and b). From the photographs, it is also clear that the Zn NPs formed are in crystalline and hexagonal shape with an average particle diameter of 20-25 nm, which is well matched with the results obtained from SEM and FESEM, analyses.

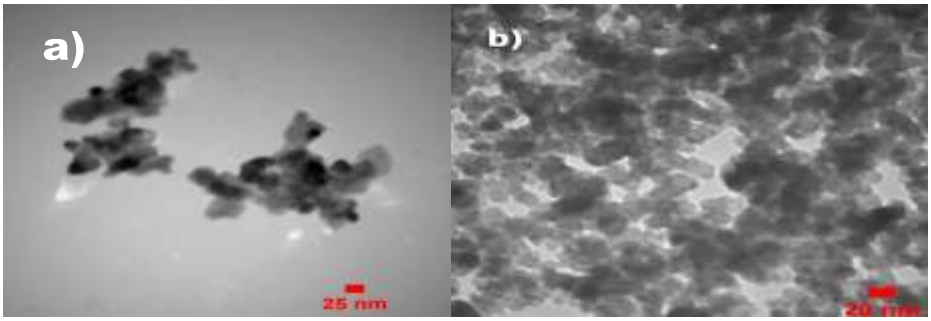
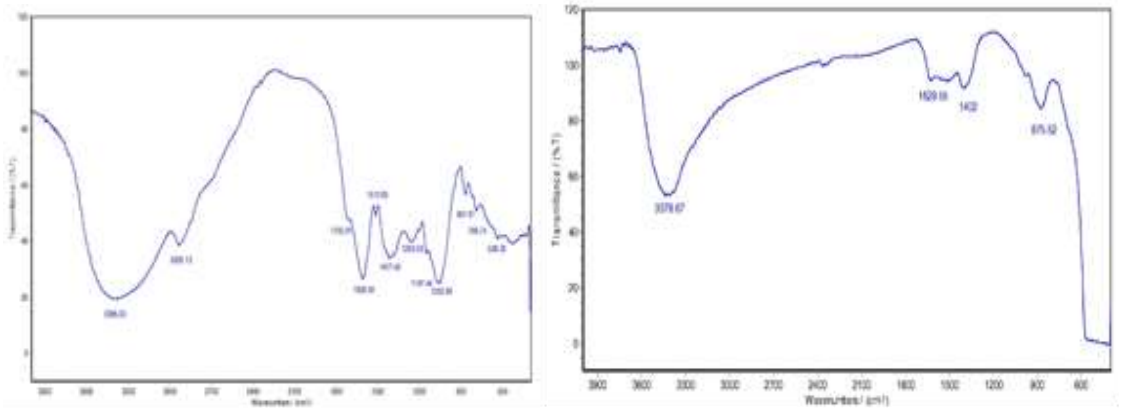


Figure 6. a,b) Transmission electron microscopy (TEM) of Zn nanoparticles synthesized by *Taxus baccata* extract

Fourier transform infrared spectroscopy (FTIR)

The chemical composition of the prepared sample was studied by FTIR spectrometry (Rayleigh WQF-510A spectrometer). The spectra were recorded in the range of 4000–400 cm-1 using KBr pellet method. The functional groups for capping as well as the efficient stabilization of the synthesized metallic nanoparticles were determined using FTIR spectrum. FTIR spectrums of Zn nanoparticles and *Taxus baccata* extract (Figure 7, a and b) showed two peaks in the regions between 3412 cm^{-1} and 2849 cm^{-1}, which were assigned to O-H stretching of alcohol and phenol compounds and aldehyde -C-H- stretching of alkanes. Peaks around the regions 1597 cm^{-1} to 1508 cm^{-1} and 1385 cm^{-1} to 827 cm^{-1} corresponded to N-H(bond) of primary and secondary amides and -C-N- stretching vibration of amines or -C-O stretching of alcohols, carboxylic acids, ethers, and anhydrides. The peaks at 600-400 cm^{-1} and 846 cm^{-1} represent the formation of metallic bond due to the presence of Zn NPs [68- 71]. The peaks at 400 to 500 cm^{-1} were observed as a result of Zn-O vibrations of Zn nanoparticles [72-75]. Maximum peaks at 1627.24, 3416.92 cm^{-1} corresponded to C=O stretching and O–H stretching of organic compound. The analysis of Zn nanoparticles using FTIR demonstrated that these organic molecules might have captured them [76]. Having compared the two spectra, it was found that the absence of some peaks or the presence of some new ones in the spectrum extracted after the synthesis of nanoparticles was a true evidence indicating the implementation of reducing agents and nanoparticles formation. Therefore, the synthesized nanoparticles were surrounded by proteins and metabolites such as alkaloids with functional groups, as shown in Figure 2. The FTIR results confirmed that carbonyl groups showed more capability of binding as compared to the amino acids and proteins groups. On the other hand, proteins that were present in the extract (i.e.; capping of Zn nanoparticles) could possibly prevent the agglomeration of metallic nanoparticles. This suggests that in an aqueous medium, the biological molecules could make dual functions of stabilization and formation of Zn nanoparticles. In the absence of other strong ligating agents with sufficient concentrations, alkaloids could be adsorbed on the metallic nanoparticles surface, possibly through interaction with carbonyl groups or π-electrons



a) **b)**

Figure 7. FT-IR spectra of *Taxus baccata* extract (a) Zn nanoparticles synthesized by *Taxus. baccata* extract(b)

Antibacterial activity effects

The antimicrobial activity of the *Taxus baccata* extract, zinc nitrate solution and Zn nanoparticles (Zn NPs) were carried out by the well diffusion method according to the protocol as discussed in the previous section. The solutions did not show antimicrobial effects against staphylococcus aureus (a gram positive bacteria), escherichia coli and pseudomonas aeruginosa (gram negative bacteria). In negative control wells, no bacterium was grown while in positive control wells, a very good growth of bacteria was observed. MIC test was performed against the three mentioned bacteria and the sample was incubated at 370 C for 24 h. To confirm the results of the test, each bacterial test was repeated four times. Table 1 shows in details MIC measurements as calculated using OD method. OD is an abbreviation indicating the absorbance and optical density of a sample and is a common method for estimating the concentration of bacterial or other cells in a liquid.

Assessment of anticancer activity by MTT assay

Zn is an important co-factor in various cellular mechanisms and plays an important role in maintaining cellular homeostasis [77]. It is the co-factor of over 300 mammalian enzymes and also has an important role against the initiation and progression of cancer in the host defense system [78]. Cells can be regularly checked by the tumor suppressor p53 gene and caspase enzyme, so Zn prevents cells to change to cancer cells. When a cell exhibits malignancy, a DNA repair mechanism is activated to repair this change. If this method cannot repair the damaged DNA, the cell undergoes 'programmed cell death' (apoptosis) to prevent the cell proliferation, which may develop into the cancerous cell. In different ways, Zn can protect cells against cancer. To evaluate the anti-cancer activity of Zn nanoparticles synthesized using the T. baccata extract, different dilutions of the nanoparticles were tested on cancer cells. Figure 8 shows the effect of the extract and nanoparticles, which is similar in reducing the average number of cells; and the tick diluted of them is indicative of the more anti-cancer effect they have on the cells. The effect of the extract and nanoparticles only led to about 22% of the cells surviving. Zinc nitrate solution in all dilutions inhibited cell growth. Its effect was about 80% of the deterrent. However, in very low dilutions (0.75 mM), the power of its effect was greatly reduced. The inhibitory activity of *T. baccata* extract, Zinc nitrate and Zn NPs were demonstrated in Table 2.

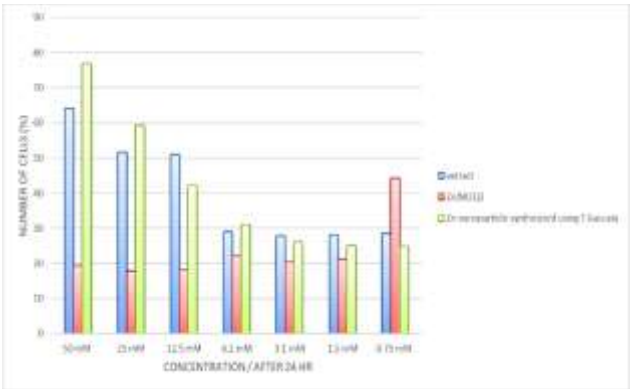


Figure 8. MTT assay for the zinc nanoparticles, extract containing Taxol and zinc nitrate solution using the *Breast* cell line

Table 1. Results of MIC studies of Zn nanoparticles and standard antibiotic against test organisms.

No	Organism	Sample	MIC (mM)
1	Staph. aureus	zinc nanoparticle Synthesized with *T. baccata* extract	25 mM (74mg $Zn(NO_3)_2$ / 10ml)
		zinc nitrate	25 mM (74mg $Zn(NO_3)_2$ / 10ml)
		T. baccata extract	0.0 (2g/10ml)
		Positive control	0.900 ± 0.05 SMP
2	E. coli	zinc nanoparticle Synthesized with *T. baccata* extract	50 mM (148mg $Zn(NO_3)_2$ / 10ml)
		zinc nitrate	50 mM (148mg $Zn(NO_3)_2$ / 10ml)
		T. baccata extract	0.0 (2g/10ml)
		Positive control	0.863 0.05 SMP
3	P. aeruginosa	zinc nanoparticle Synthesized with *T. baccata* extract	25 mM (74mg $Zn(NO_3)_2$ / 10ml)
		zinc nitrate	25 mM (74mg $Zn(NO_3)_2$ / 10ml)
		T. baccata extract	0.0 (2g/10ml)
		Positive control	0.882 0.05 SMP

Table 2. Breast cell proliferation inhibitory activity of *Taxus baccata* extract, zinc nitrate and Zn NPs.

The studied solutions	No.	Concentration of zinc nitrate (mM)	Dilutions	%Cell viability	%Cell inhibition
Zn NPs synthesized by T. *baccata* extract	1	0.75	1/64	76.94	23.06
	2	1.5	1/32	59.31	40.69
	3	3.1	1/16	42.26	57.74
	4	6.25	1/8	31.15	68.85
	5	12.5	1/4	26.03	73.97
	6	25	1/2	25.03	74.97
	7	50	1/1	24.83	75.17
Zinc nitrate	1	0.75	1/64	44.23	55.77
	2	1.5	1/32	21.05	78.95
	3	3.1	1/16	20.48	79.52
	4	6.25	1/8	22.19	77.81
	5	12.5	1/4	18.2	81.8
	6	25	1/2	17.78	82.22
	7	50	1/1	19.2	80.8
T. *baccata* extract	1	0	1/64	64.15	35.85
	2	0	1/32	51.63	48.37
	3	0	1/16	46.92	53.08
	4	0	1/8	29.01	70.99
	5	0	1/4	27.88	72.12
	6	0	1/2	28.16	71.84
	7	0	1/1	28.45	71.55

Conclusion

In this study, the biosynthesis of Zn nanoparticles by *Taxus baccata* extract was studied using UV-Vis spectrophotometry in the range of 250 to 400 nm. In order to achieve optimal synthesis conditions, effective parameters were investigated. As it is observed in Figure 4 (a-e), the basic evaluation of nanoparticle was carried out using surface plasmon resonance (SPR), where the higher peak showed the formation of more nanoparticles. Zn NPs were characterized by UV–Vis spectroscopy under different parameters

of pH=7, volume extract of 2 mL, 0.0025 M $Zn(NO_3)_2$, t=25 °C and time=24 h.
In optimum conditions, Zn nanoparticles with the sizes of 20 to 25 nanometers were synthesized. The results obtained from UV-Vis spectrophotometry, SEM, FESEM and TEM analyses confirmed the efficiency of *Taxus baccata* leaves extract for the synthesis of high pure crystalline Zn nanoparticles. Also, FTIR spectrum of the extract showed the role of functional groups in zinc ions reduction. So, according to these results, the synthesis of metallic nanoparticles using herbal extracts is a good alternative for chemical and physical methods. Also, Zn nanoparticles, *Taxus baccata* leaves extract and zinc nitrate solution were tested for antimicrobial activity against Staphylococcus aureus, escherichia coli and pseudomonas aeruginosa and as a result, Zn nanoparticles did not show any antibacterial activity. This report suggests that the biosynthesized Zn NPs using *Taxus baccata* leaves extract have cytotoxic effect on human breast cancer cell line MCF-7. However, in vitro cytotoxicity effect on breast cancer cell line proved the potential application of Zn NPs synthesized through the *Taxus baccata*. The MTT test by MCF-7 Breast cancer cell line proved that the anti-cancer activity of Zn nanoparticles was expected to be higher than that of extract alone. In this study, it was found that Zn nanoparticles could be synthesized by green chemistry route without any harmful chemicals and it had not any effect on bacteria (gram positive and gram negative). The investigation of anticancer activity extract containing Taxol mixed with zinc nitrate solution confirmed that Zn nanoparticles had very high anti-cancer activity.

Acknowledgements

The authors gratefully acknowledge financial support from the Arak Branch, Islamic Azad University, Arak, Iran.

References

[1] P.V. Rao, SH. Gan, *Curr Drug Metab*, **2015**, *16*, 371-375.
[2] M. Noruzi, *Bioprocess Biosyst. Eng.*, **2015**, *38*, 1-13.
[3] D. Suresh, R.M. Shobharani, P.C. Nethravathi, M.A. Pavan-Kumar, H. Nagabhushana, *Spectrochim Acta A Mol Biomol Spectrosc*, **2015**, *141*, 128-134.
[4] H. Duan, D. Wang, Y. Li, *Chem. Soc. Rev.*, **2015**, *44*, 78-92.
[5] S.S. Ashtaputre, A. Deshpande, S. Marathe, M.E. Wankhede, J. Chimanpure, R. Pasricha, J. Urban, S.K. Haram, S.W. Gosavi, S.K. Kulkarni, *Pramana J. Phys.*, **2005**, *65*, 615-620.
[6] R. Wahab, Y.S. Kim, D.S. Lee, J.M. Seo, H.S. Shin, *Sci. Adv. Mater.*, **2010**, *2*, 35-42.
[7] H. Bar, D.K. Bhui, G.P. Sahoo, P. Sarkar, S. Pyne, A. Misra, *Colloids Surf Physiochem Eng Aspects*, **2009**, *348*, 212-216.
[8] K. Prasad, A.K. Jha, *Jha. Nat. Sci*. **2009**, *1*, 129-135.
[9] G. Sangeetha, S. Rajeshwari, R. Venckatesh, *Mater Res Bull*, **2011**, *12*, 2560-2566.
[10] C. Jayaseelan, A. Abdul Rahuman, A. Vishnu Kirthi, S. Marimuthu, T. Santhoshkumar, A. Bagavan, K. Gaurav, L. Karthik, K.V. Bhaskara Rao, *Spectrochimica Acta. Part A.*, **2012**, *90*, 78-84.
[11] R. Chandrasekaran, S. Gnanasekar, P. Seetharaman, R. Keppanan, W. Arockiaswamy, S. Sivaperumal, *J. Mol. Liq.*, **2016**, *219*, 232-238.
[12] M. Sundrarajan, S. Ambika, K. Bharathi, *Adv. Powder Technol*. **2015**, *26*, 1294-1299.
[13] T.C. Taranath, B.N. Patil, *Int J Mycobacteriology*, **2016**, *5*, 197-204.
[14] P. Jamdagni, P. Khatri, J.S. Rana, *J King Saud Univ Sci.*, **2018**, *30*, 168-175.
[15] J. Pulit-prociak, J. Chwastowski, A. Kucharski, M. Banach, *Appl Surf. Sci.*, **2016**, *385*, 543-553.

[16] H. Mirzaei, M. Darroudi, *Ceram Int.*, **2017**, *43*, 907-914.
[17] S. Jiang, Y. Zhang, Y. Zu, Z. Wang, Y. Fu, *Am. J. Chin. Med*., **2010**, *38*, 1107–1114
[18] K. Miller, B. Neilan, D.M. Sze, *Recent Pat. Anticancer Drug Discov*, **2008**, *3*, 14–19
[19] Y. Li, F. Qin, S.M. Wang, R.X. Guo, Y.F. Zhang, Y.C. Gu, Q.W. Shi, *Chem. Biodivers*, **2013**, *10*, 1729–1753
[20] S.S. Yu, Q.W. Sun, X.P. Zhang, S.N. Tian, P.L. Bo, *Ying Yong Sheng Tai. Xue. Bao.*, **2012**, *23*, 2641–2647
[21] J. Zhang, Q.J. Shu, J.L. Gao, L. Zhang, *Zhongguo Zhong Xi. Y.i Jie. He. Za. Zhi*., **2013**, *33*, 805–809
[22] J. Kobayashi, H. Shigemori, *Med. Res. Rev.*, **2002**, *22*, 305–328
[23] S.H. Hoke, J.M. Wood, R.G. Cooks, X.H. Li, C.J. Chang, *Anal. Chem.*, **1992**, *64*, 2313–2315
[24] Z. Liang, Z. Xie, S. Lam, X. Xu, *J. Sep. Sci.*, **2014**, *37*, 2322–2330
[25] Z.K. Liang, R.G. Huang, Z.S. Xie, X.J. Xu, *Nat. Prod. Res*., **2014**, *29*, 1–4
[26] Y. Zu, Y. Fu, S. Li, R. Sun, Q. Li, G. Schwarz, *J. Sep. Sci.*, **2006**, *29*, 1237–1244
[27] S. Li, Y. Fu, Y. Zu, R. Sun, Y. Wang, L. Zhang, H. Luo, C. Gu, T. Efferth, *J. Pharm. Biomed. Anal.*, **2009**, *49*, 81–89
[28] S. Li, Y. Fu, Y. Zu, B. Zu, Y. Wang, T. Efferth, *J. Sep. Sci.*, **2009**, *32*, 3958–3966
[29] C. Yang, Y. Xiao, Z. Wang, S. Wang, L. Chen, L. Wu, G. Liu, *J. Chromatogr. B.*, **2014**, *960*, 59–66
[30] H. Luo, Y.K. Nie, Y.J. Fu, Y.G. Zu, S.M. Li, W. Liu, L. Zhang, M. Luo, Y. Kong, Z.N. Li, *J. Sep. Sci.*, **2009**, *32*, 192–201
[31] Z. Salari, A. Ameri, H. Forootanfar, M Adeli-Sardou., M. Jafari, M. Mehrabani, M. Shakibai, *Journal of Trace Elements in Medicine and Biology.*, **2017**, *39*, 116-123.
[32] I. Sheikhshoaie, Z. Tohidiyan, *Chem. Methodol.*, **2019**, *3*, 30-42.
[33] I. Sheikhshoaie, M. Sheikhshoaei, S. Ramezanpour, *Chem. Methodol.*,**2018,** *2*,103-113.
[34] S. Sajjadifar, Z. Arzehgar, A. Ghayuri, *J. Chin. Chem. Soc.*, **2018**, *65*, 205-211.
[35] S. Sajjadifar, Z. Arzehgar, S. Khoshpoori, *J. Inorg. Organomet. Polym. Mater.*, **2018**, *28* , 837-846.
[36] I. Sheikhshoaie, S. Davari, S. Ramezanpour, *Chem. Methodol.*, **2018**, *2*, 47-55.
[37] O. Solomon, W. Rabiu-Saidu-Umar, H. Sanusi-Wara, A. Sadiq-Yakubu, M. Michael-Azubuike, M. Asugu-Mary, H. Louis, *Prog. Chem. Biochem. Res.*, **2018**, 0*1*(1, 1-59), 29-39.
[38] A.A. Revina, E.V. Oksentyuk, A.A. Fenin, *Prot Met.* **2007**, *43*, 554-559.
[39] A. Mirzaie, *J. Med. Chem. Sci.*, **2018**, *1*, 5-8.
[40] R. Mohammadi, A. Sajjadi, *J. Med. Chem. Sci.*, **2019**, *2*, 55-58.
[41] F. Ghandehari, M. FaniMalahat, *J. Med. Chem. Sci.*, **2018**, *1*, 28-30.
[42] A. Hameed, G.R. Fatima, K. Malik, A. Muqadas, M. Fazal-ur-Rehman, *J. Med. Chem. Sci.*, **2018**, *2*, 9-16.
[43] A.A.P. Almeida, A. Farah, D.A.A. Silva, E.A. Nunan, M.B. Glória, J. Agric, *Food Chem.*, **2006**, *54*, 8738-8743.
[44] F. Mohammadi, M. Yousefi, R. Ghahremanzadeh, *Adv. J. Chem. A*, **2019**, *2*, 266-275.
[45] K.S. Sindhura, T.N.V.K.V. Prasad, P.P. Selvam, O.M. Hussain, *Appl Nano sci.*, **2014**, *4*, 819-827.
[46] J. Qu, X. Yuan, X. Wang, P. Shao, *Environmental Pollution.* **2011**, *159*, 1783-1788.
[47] P. Rajiv, S. Rajeshwari, R. Venckatesh, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **2013**, *112*, 384-387.
[48] E. Preedia Babu, A. Subastri, A. Suyavaran, P. Lokeshwara Rao, M.

Suresh Kumar, K. Jeevaratnam, C. Thirunavukkarasu, *RSC Adv.*, **2015**, *5*, 62067- 62077.
[49] E. Preedia Babu, A. Subastri, A. Suyavaran, K. Premkumar, V. Sujatha, B. Aristatile, G.M. Alshammari, V. Dharuman, C. Thirunavukkarasu, *Scientific Reports.*, **2017**, *7*, 4203.
[50] F. Malek Mohammadi, N. Ghasemi, *J. Nanostruct. Chem.***2018**, *8*, 93–102.
[51] K. Sri Sindhura, T.N.V.K.V. Prasad, P. Panner Selvam, O.M. Hussain, *App,l Nanosci*,, **2014**, *4*, 819–827.
[52] A.A. Revina, E.V. Oksentyuk, A.A. Fenin, *Prot. Met.*, **2007**, *43*, 613–618.
[53] D.K. Badarinath, S. Samim, B. Mayuri, D. Ishita, M.P. Padmaa, A.B. Vedamurthy, *Phcog. J.*, **2016**, *8*, 127-131.
[54] b. Wesam Salema, R. Deborah, 1. Leitnera, G. Franz, 1. Zingla, S. Gebhart, R. Prasslc, W. Goesslerd, J. Reidla, S. Schilda, *Int. J. Med. Microbiol.*, **2015**, *305*, 85–95.
[55] G. Sangeetha, S. Rajeshwari, R. Venckatesh, *Mater. Res. Bull.* **2011**, *46*, 2560-2566.
[56] S. Gunalan, R. Sivaraj, V. Rajendran, *Int. Mater.*, **2012**, *22*, 693-700.
[57] F.T. Thema, E. Manikandan, M.S. Dhlamini, M. Maaza, *Material Letters*, **2015**, *161*, 124-127.
[58] L.F.A. Anand Raj, E. Jayalakshmy, *Orient. J. Chem.*, **2015**, *31*, 51-56.
[59] N.P. Bheemanagouda, C.T. Tarikere, *Int. J. Mycobacteriol.*, **2016**, *5*, 197-204.
[60] G. Bisht, S. Rayamajhi, *Nanobiomedicine*, **2016**, *3*, 3-9.
[61] S. Saranya, A. Eswari, E. Gayathri, S. Eswari, K. Vijayarani, *Int. J. Curr. Microbiol. App. Sci.*, **2017**, *6*, 1834–1845.
[62] A. Sadollahkhani, I. Kazeminezhad, J. Lu, O. Nur, L. Hultman, M. Willander, *RSC Adv.*, **2014**, *4*, 36940–36950.
[63] E., George, *Int. J. Adv. Res. Sci. Eng.*, **2015**, *4*, 307–314.
[64] F. Namvar, S. Azizi, H.S. Rahman, R. Mohamad, A. Rasedee, M. Soltani, R.A. Rahim, *Onco Targets Ther*., **2016**, *9*, 4549–4559.
[65] T. Kaviyarasi, B. Muthulakshmi, C. Kavitha, *Int. J. Chem. Technol. Res.*, **2017**, *10*, 344–348.
[66] J. Zhou, N.S. Xu, Z.L. Wang, *Adv. Mater.*, **2006**, *18*, 2432-2435.
[67] E. Ho, Zinc deficiency, *J. Nutr. Biochem.*, **2004**, *15*, 572-8.
[68] S.N. Sinha, D. Paul, *Spectrosc. Lett.*, **2015**, *48*, 600-604.
[69] Z. Oruç, M. Ergüt, D. Uzunoğlu, A. Özer, *J. Environ. Chem. Eng.*, **2019**, *7*, 103231.
[70] T. Zhu, T. YuWu, J. AoRen, S. JianQian, Y. Li, W. TingSu, D. FengZou, Q. YunLi, J. Yang, *Inorganica Chim. Acta*, **2019**, *487*, 70-75.
[71] N. Pauzi, N. MatZain, N.A. AhmadYusof, *Proceedings of the 10th* ***National Technical Seminar on Underwater System Technology*****, 2018, *538*, 541-546.**
[72] M. Geravand, F. JamaliSheini, *Adv. Powder Technol.*, **2019**, *30*, 347-358.
[73] P.T. Phong, P.H. Nam, N.X. Phuc, B.T. Huy, L.T. Lu, D.H. Manh, In-Ja Lee, *Metall. Mater. Trans.*, **2019**, *50*, 1571–1581.
[74] H.BinduDuvuru, S.K. Alla, S.K.Shaw, Sh. SinghMeena, N. Gupta, B.B.V.S. VaraPrasad, M.M. Kothawale, M.K. Kumar, N.K. Prasad, *Ceram. Int.*, **2019**, *45*, 16512-16520.
[75] N.D. Krupa, A.N. Grace, V. Raghavan, *Institu. Eng. Technol.*, **2019**, *13*, 510 – 514.
[76] H.Y. Chai, S.M. Lam, J.Ch. Sin, *Mater.Letters*, **2019**, *242*, 103-106.
[77] G.Sharmila, M. Thirumarimurugan, Ch. Muthukumaran, *Microche. J.*, **2019**,*145*, 578-587.

[78] S. TaghaviFardood, A. Ramazani, F. Moradnia, Z. Afshari, S. Ganjkhanlu, F. YekkeZare, *Chem. Methodol*., **2019**, DOI: 10.33945/SAMI/CHEMM.2019.6.1.

How to cite this manuscript: Sona Sarli, Nahid Ghasemi. Optimization of biosynthesized Zn nanoparticles by poisonous *Taxus baccata* leaves extract and evaluation of their effect on the bacterias and MCF-7 cancer cells. *Eurasian Chemical Communications*, 2020, 2(3), 302-318.