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# GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING AQUEOUS EXTRACT OF *ACHIELLA MILLEFOLIUM L.*: IN VITRO ANTI-CANCER POTENTIAL ON LUNG AND COLON CANCER CELLS

Çiğdem Aydın ACAR*[a]

[a]*Burdur Mehmet Akif Ersoy University, Bucak School of Health, Department of Nursing, Burdur, Turkey*

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*Correspondence: Cigdem Aydin Acar
Burdur Mehmet Akif Ersoy University, Bucak School of Health, Department of Nursing, Burdur, Turkey
e-mail: cacar@mehmetakif.edu.tr

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**ABSTRACT**

In recent years, zinc oxide nanoparticles (ZnONPs) synthesized using plants have become an interesting field especially in nanomedicine applications due to their high biocompatibility and stability. In the present study, ZnONPs were synthesized using aqueous extract *of Achiella millefolium L.* Characterization of the green synthesized ZnONPs were carried out using UV vis spectroscopy. Cytotoxic activity on A549 lung cancer cells and HT29 colon adenocarcinoma cells was evaluated by MTT Assay. The UV–vis spectroscopy result revealed an absorption peak in the range of 356 nm. The green synthesized ZnONPs showed significant cytotoxic effects on A549 and HT29 cell lines in a dose-dependent manner. The obtained IC50 value of A549 cells were 46.47 while, IC50 value of HT29 cells were 42.82 µg/mL for ZnONPs. ZnONPs had a similar cytotoxic activity on A549 and HT29 cells. In conclusion, ZnONPs were synthesized through a simple, cost effective and eco-friendly green route via the use of *Achiella millefolium L.* extract. The green synthesized ZnONPs showed strong cytotoxic activity against lung and colon cancer cell lines.

## INTRODUCTION

Today, nanotechnology attracts increasing attention in its applications in science and industry. Nanotechnology is the characterization, production and application of 1-100 nanometer sized nanoparticles. The word nano is a Greek word synonymous with dwarf. Nanotechnology is a rapidly growing field that has emerged to produce new materials at the nanoscale with advances in science and technology (1). With this technology at the nanoscale level; It is ensured that materials with different electrical, magnetic, optical, physical and chemical properties are formed, depending on the surface area/volume ratio, modified for human benefit (2). Nanoparticles can be synthesized using many materials with different chemical properties. However, metals, metal oxides, biomolecules, silicates, polymers, carbon and organic molecules are materials commonly used to synthesize nanoparticles. Nanoparticles are collected in two groups as organic and inorganic. Inorganic nanoparticles contain semiconductor nanoparticles (such as ZnO, ZnS, CdS), metallic nanoparticles (such as Au, Ag, Cu, Al) and magnetic nanoparticles (Co, Fe, Ni), organic nanoparticles contain carbon particles (quantum dots, carbon nanotubes) (3).

Zinc oxide (ZnO) nanoparticles are one of the favorite study subjects of researchers due to their optical and electrical properties (4-7). Various activity studies are carried out after synthesis for nanoparticles used in many fields. Zinc oxide nanoparticles (ZnO NPs) are known to inhibit the growth of various bacterial strains

and have a cytotoxic effect against various cancer cell lines (8-10). ZnO NPs are described as excellent drug delivery systems. The US Food and Drug Administration (FDA) supports the use of ZnO NPs with a particle size greater than 100 nm as a drug delivery system and reports that these particles are biocompatible (9). Compared to other metal oxide nanoparticles, ZnO is relatively inexpensive, biocompatible and less toxic, which has been supported by studies that have a wider application potential (11, 12). In addition, it has been shown that ZnONPs do not interact with most of the pharmaceutically active molecules (13).

Various methods are used for the synthesis of nanoparticles. Among these methods, biological method is preferred more than physical and chemical methods because of its environmentally friendly and economical nature (14). In the synthesis of zinc oxide nanoparticles, the use of plant sources has recently attracted more attention since it is more economical and application processes are easier. ZnONPs obtained by green synthesis are also important for live applications, with the feature of being biocompatible. Biological sources containing various natural molecules such as plants, bacteria, fungi, algae are used for the green synthesis of nanoparticles (15-18). Previous studies have suggested that plants are a good candidate because their synthesis rate is faster than other organisms and is more suitable for large-scale nanoparticle biosynthesis. In addition, the nanoparticles produced through plants are more various in shape and size in comparison with those produced by other organisms such as bacteria, fungi and algae (19). It is also known that many bioactive components in plants such as alkaloids, terpenoids, favonoids, amino acids, enzymes, vitamins, proteins and glycosides are involved in the bio-reduction, formation and stabilization of nanoparticles (20-22).

*Achillea millefolium L.* is a member of the Asteraceae family, commonly known as "yarrow". *Achillea millefolium L.* is represented by about 85 species commonly found in Europe and Asia and is among the plant species commonly used in traditional medicine. *Achillea millefolium L.* has been used in many different cultures from Europe to Asia for spasmodic gastrointestinal disorders, gynecological diseases, inflammation and wound healing. Phenolic compounds such as flavonoids and phenol carbonic acids constitute one of the most important groups in *Achillea millefolium L.* (23-26). Until now, biosynthesis of ZnONPs using *Achillea millefolium L.* extract has not been reported.

In this study, after characterization of ZnONPs synthesized with the plant extract of yarrow (*Achillea millefolium L.),* their anti-cancer effects were investigated.

## MATERIALS AND METHOD

### *Materials*

Dulbecco's Modified Eagle's Medium (DMEM), L-glutamine, Fetal bovine serum (FCS), Penicillin-Streptomycin solution and Trypsin- EDTA solution were obtained from Biowest (France). Dimethyl sulfoxide, 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Zinc acetate dihydrate [Zn(CH3COO)2.2H2O] were provided from Sigma-Aldrich (St. Louis, Missouri, USA).

### *Preparation of Achiella millefolium L. extract*

Dried *Achiella millefolium L.* was purchased from local market. Aqueous extract of *Achiella millefolium L.* was prepared in accordance with the method suggested by Aydin Acar et al. (27). 2 g of dried *Achiella millefolium L.* were boiled in 100 ml of deionized water in a microwave oven for 2 minutes. After the prepared aqueous extract was cooled, it was filtered through whatman no.1 filter paper and used fresh in the green synthesis stage.

### *Green synthesis and characterization of ZnONPs*

ZnONPs were synthesized using the method suggested by Donmez (28). For this, $Zn(CH_3COO)_2.2H_2O$ was dissolved in 50 mL double distilled water so that the final concentration was 0.02M. Next, 20 mL of *Achiella millefolium L.* aqueous extract was added to the $Zn(CH_3COO)_2.2H_2O$ solution. The resulting mixture was heated at 80 °C for 2 hours with

continuous stirring in a magnetic stirrer until a white-yellow precipitate was formed. The ZnONPs precipitate were centrifuged at 10,000 rpm for 10 minutes and washed with distilled water and then methanol. ZnONPs was dried at 60˚C overnight.

The UV ultraviolet-visible spectroscopy (Shimadzu UV-1801) was used for characterization of synthesized ZnONPs. The absorbance spectra were recorded at a wavelength of 250-500 nm.

***Cell Culture***

The A549 (human lung carcinoma) and HT29 (human colon adenocarcinoma) cell line were chosen to evaluate the cytotoxicity of ZnONPs. A549 and HT29 cells were grown in complete DMEM containing 5% fetal calf serum with 1% penicillin-streptomycin solution at 37˚C in 5% CO2 atmosphere. Passaging was performed by removing the grown cells with the help of 0.05% Trypsin-EDTA. The morphological changes of cells were followed and photographed under an inverted microscope (Olympus CK40).

***In vitro cytotoxicity assay***

The *in vitro* cytotoxicity of green synthesized ZnONPs on A549 and HT29 cells was evaluated by using 3-(4,5-dimethylthiazol-2-yl)-2,5- dephenyltetrazolium bromide (MTT) assay. Briefly, cells were seeded at a density of 5000 cells per well in 96-well microplates and incubated for 24 h for attachment of cell lines to the plate. Various concentrations of ZnONPs in medium were prepared and added to the cultured cells (0-400 µg/mL). A549 and HT29 cells not treated with ZnONPs were used as a control group. After 72 hours of incubation, 40 µL of a 5 mg/mL MTT solution was added to each well and incubated for a further 4 h at 37ºC in the dark. Afterwards, the medium was aspirated and the remaining purple formazan was lysed with 100 µL dimethyl sulfoxide. After incubation, cell viability was measured spectrophotometrically at 595 nm (Microplate reader, Biotech Inc., USA). The results were given as the means of three independent experiments. The cell viability was calculated using the following equation:

Cell viability (%) = (Absorbance of Sample/ Absorbance of Control) x100

The 50% inhibitory concentration (IC50) value, defined as the amount of sample that inhibits 50% of cell growth was determined from the absorbance versus concentration curve. Calculation was done using AAT Bioquest IC50 calculation tool. Data were then analysed using the Student's *t*-test and considered significant if $p < 0.05$.

## RESULTS AND DISCUSSION

***Synthesis and UV-visible Analysis of ZnONPs***

In this study, the *green ZnO*NPs were successfully synthesized using aqueous extract of *Achiella millefolium L.* Figure 1 represents the green synthesis of ZnONPs using *Achiella millefolium L.* extract. Visual color change is the preliminary test for zinc oxide

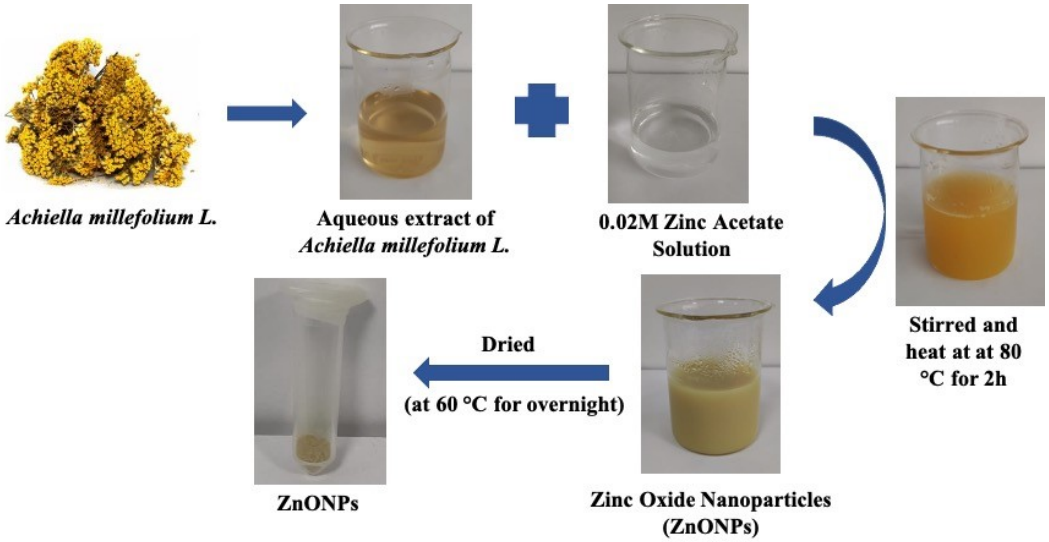


**Figure 1.** Green Synthesis of ZnONPs using *Achiella millefolium L.* extract

nanoparticle synthesis. Color change from orange to pale yellow represents the synthesis of ZnO NPs.

UV-vis spectroscopy analysis is the most significant analysis to confirm the formation and stability of ZnONPs. The formation of the zinc oxide nanoparticles was confirmed by UV–vis spectroscopy within the wavelength range of 250–500 nm. The absorption spectrum of green synthesized ZnONPs showed a characteristic peak at 356 nm, confirming the formation of ZnO nanoparticles (Figure 2). This result is similar to findings previously reported by Safawo et al. (29) and Donmez (28).

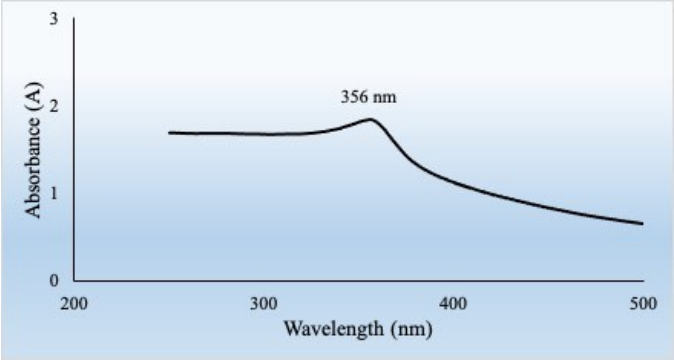


**Figure 2.** UV Spectrum of synthesized zinc oxide nanoparticles

***Cytotoxic effects of the green synthesized ZnONPs on lung and colon cancer cells***

The cytotoxic effects of ZnONPs from the *Achillea millefolium L.* extract on A549 human lung carcinoma and HT29 human colon adenocarcinoma cells were evaluated after 72 h treatment with different nanoparticle concentrations (0 and 400 µg/ml) using MTT assay. The MTT assay results given in Figure 3 showed that ZnONPs had a pronounced cytotoxic effect on A549 and HT29 cells compared to the control group. Also, these results indicated that the cytotoxic effect ZnONPs was dose dependent manner. As shown in Figure 3, ZnONPs had a similar cytotoxic activity on A549 and HT29 cells. The obtained IC50 value of A549 cells were 46.47 while, IC50 value of HT29 cells were 42.82 µg/mL for ZnONPs.

Microscopic studies of A549 and HT29 cells showed morphological changes following with the suppression of cell growth and nally the cell clumping and death due to the exposure to the zinc oxide nanoparticles (Figure 4).

There are a limited number of studies on the cytotoxic effects of green synthesized ZnONPs using plant extract on lung cancer cells. Rajeshkumar et al. (30) evaluated the antioxidant and cytotoxic effects of ZnONPs biosynthesized from mango leaves on A549 cells and compared their effects with low dose cyclophosphamide and mango leaves extract. They emphasized that the concentration of nanoparticle applied plays a very important role in anti-cancer properties. Vijayakumar et al. (31) reported the cytotoxic effects of zinc oxide nanoparticles

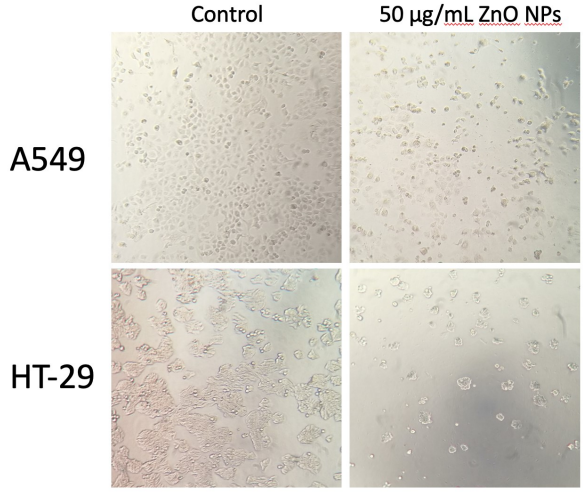


**Figure 3.** Cytotoxic effect of green synthesized ZnONPs for 72 h, on the growth of A549 and HT29 cells determined by MTT assay

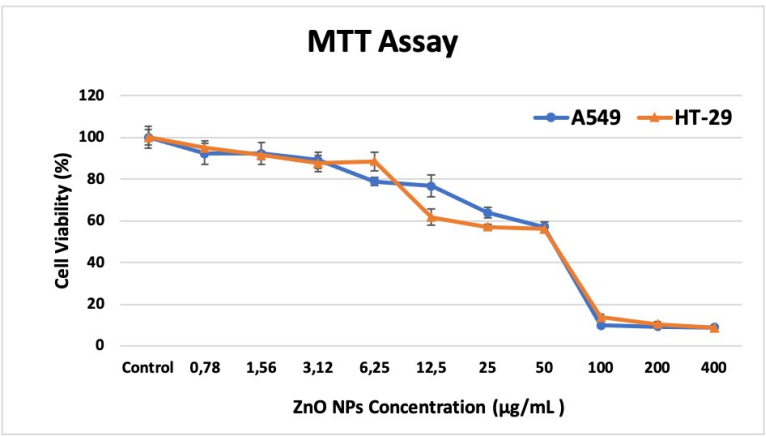


**Figure 4.** Morphological changes of A549 and HT29 cells after incubation ZnONPs for 72 hours

synthesized by green synthesis using aqueous *Laurus nobilis* leaf extract on normal murine RAW264.7 macrophage cells and A549 lung cancer cells. The cytotoxicity studies revealed that ZnO NPs showed no effect on normal murine RAW264.7 macrophage cells. On the other hand, ZnO NPs were effective in inhibiting the viability of human A549 lung cancer cells at higher concentrations of 80 mg/mL. A study on the cytotoxic effects of ZnONPs synthesized by green synthesis on HT29 colon cancer cells has not been found in the literature. However, there are studies evaluating the effects of ZnONPs synthesized by different methods on HT29 cancer cells. In these studies, in accordance with our study, ZnONPs have been shown to reduce cell proliferation in HT29 colon cancer cells depending on the dose (32-35).

## CONCLUSION

In conclusion, a simple, cost effective and green method was applied in the synthesis of ZnONPs using *Achiella millefolium L*. extract in this study. In general, green methods are less toxic than chemical methods of nanoparticle synthesis. In addition, it was shown that green synthesized ZnONPs in two different cell lines (A549 and HT29), which were taken as a model for cancer, exhibited anticancer effects. The mechanisms of ZnONPs regarding their apoptotic effect on cancer cells should be elucidated. Studies on apoptotic signaling pathway which triggered by the ZnONPs in A549 and HT29 cells are needed. Finally, ZnONs prepared using *Achiella millefolium L*. extract have the potential to be considered as an effective anticancer agent, further *in vitro* and *in vivo* studies should be performed in order to use these nanoparticles in clinic.

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