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Green synthesis of ZnO nanoparticles using the aqueous extract of *Euphorbia petiolata* and study of its stability and antibacterial properties

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Abstract

Through this study for the first time the biosynthesis, identification, stability and antibacterial activity of zinc oxide (ZnO) nanoparticles (NPs) was studied using the Euphorbia petiolata Banks aqueous extract as a reducing and stabilizing media during a simple and green method. Interaction of plant extract with the aqueous mixture of zinc nitrate and the oxidation via annealing process efficiently caused the reduction of the Zn ions and formation of zinc oxide nanoparticles. The stability, purity and crystalline nature of green synthesized nanoparticles was demonstrated using Uv-vis spectroscopy, EDS and XRD techniques, respectively. Also, the scheme of possible mechanism leading to the formation of NPs was illustrated. Moreover, the efficient antibacterial ability of green synthesized nanoparticles against *Escherichia coli* was demonstrated compared to the plant extract and chloramphenicol as positive control.

Keywords: Green Synthesis; Stability; ZnO Nanoparticles; Antibacterial activity; *Euphorbia petiolata*

1. Introduction

During the recent years nanotechnology plays an important role in progress of science and technology. Among the different types of nanostructures those produced from transition metals such as metal and metal oxide nanostructures have generated considerable interest as next generation technologies [1]. Among the metal oxide nanostructures, zinc oxide (ZnO) as a multi-tasking metal oxide is one of the best metal oxides with wide applications in nanoscale technologies such as pharmaceutical and cosmetic industries, solar energy materials (solar cells), gas sensors and photo catalysts due to its unique physicochemical properties [2, 3]. Several physical and chemical techniques were used to synthesis of ZnO NPs and other nanomaterials but some of these methods suffer from high pressure and temperature, high expense, short stability and adsorption of toxic chemicals that may have adverse the medical applications [4, 5]. Currently biological methods have been attracted the attention of many researchers to biosynthesis of nanostructures due to their simplicity, non expensive, accessibility and environmentally friendly properties [6-9]. In fact the use of environmentally benign materials such as plant extracts, bacteria, fungi, and enzymes for the synthesis of nanostructures reveals numerous benefits and compatibilities for pharmaceutical and other biomedical applications on which beside the high energy inputs, toxic materials are not used for synthesis protocols and therefore no interference and contamination is observed while synthesis of nanostructures [10-13]. Among the biological sources for biosynthesis of nanostructures, plants have considerable importance due to presence of a large spectrum of phytochemicals such as antioxidant flavonoids, glycosides, vitamins, terpenoids and tannins which act as reducing agents and made the plant extracts as suitable and potential green media to produce the nanostructures [9]. Up to now, there are many reported literatures concerning the biosynthesis of ZnO NPs but for strong potential of the plants to synthesis of nanostructures and also variety of plant phytochemical contents and their actions, new researches are still being reported [14-16]. The genus *Euphorbia* (*Euphorbiaceae* or spurge family) are one of the most populated plant families with a large distribution in different parts of the world which found as herbs, shrubs and trees. In fact, plants of this genus are perennial herbaceous plant containing latex with unique flowered structures. Members of Euphorbia are rich in phenolics, aromatic esters, steroids, diterpenoids, tetracyclic triterpenoids, pentacyclic triterpenoids, essential oils and several bioactive constituents [17-19]. A chemical literature survey of *Euphorbia petiolata* revealed the presence of various range of phytochemicals especially antioxidant phenolics including both aglycones and glycosides such as quercetin, kaemferol and myricetin and those with sugar moieties were demonstrated in different parts of the plant along with their application in medicine and pharmacology [20]. These phyto-constituents confirmed the application of *Euphorbia petiolata* leaf extract as a suitable source for synthesis of nanoparticles using the reducing ability of these potent antioxidants.

Figure 1. Image of *Euphorbia petiolata* plant

Therefore, in continuation with our research program to explore different methodologies for the green synthesis of various metal NPs and their abilities and characteristics, [21-30], during this work for the first time the synthesis of the ZnO nanostructure was reported via the reduction of Zn^{+2} ions using *Euphorbia petiolata* leaf extract as a reducing and stabilizing agent, which exhibit excellent structural stability and antibacterial activity against *E.Coli*. Furthermore, the probable mechanism for the formation of ZnO NPs was reported.

2. Materials and methods

2.1. Instruments and reagents

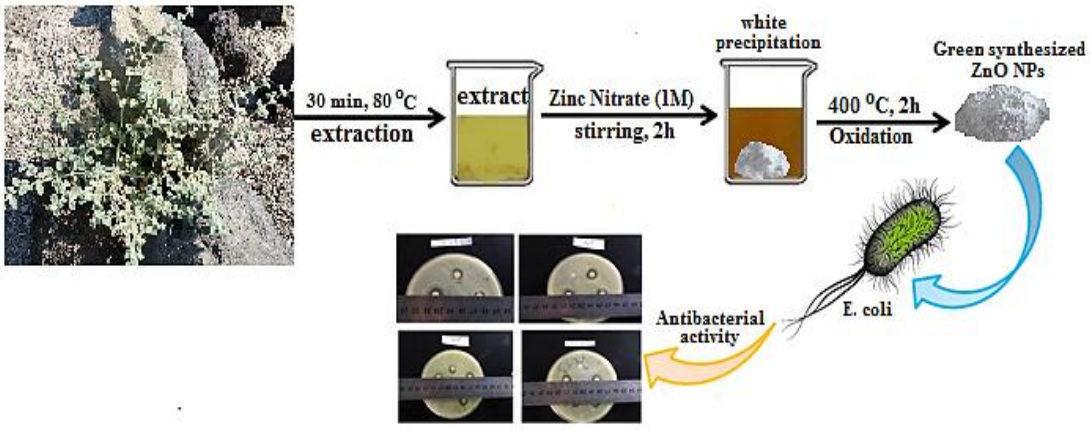
High-purity chemicals were purchased from the Merck and Aldrich chemical companies. X-ray diffraction (XRD) measurements were carried out using a Philips powder diffractometer type PW 1373 goniometer (Cu Kα = 1.5406 A˚). The scanning rate was 2º/min in the 2θ range from 0 to 90˚. UV-visible spectral analysis was recorded on a double- beam spectrophotometer (Hitachi, U- 2900) to ensure the formation of nanoparticles. Morphology and particle dispersion was investigated by fast emission scanning electron microscopy (FE-SEM) (Cam scan MV2300). The chemical composition of the prepared nanostructures was measured by EDS (Energy Dispersive X-ray Spectroscopy) performed in SEM. The antibacterial activity of green synthesis ZnO nanoparticles was studied using the disc diffusion method via Muller Hinton media and by using the Chloramphenicol as positive control. The E. Coli was obtained from the department of microbiology, Soran University in Kurdistan, Iraq.

2.2. Preparation of Euphorbia petiolata leaf extract

50 g of dried powder leaf of the plant was boiled in 500 mL double distilled water for 30 minutes at 80 °C then the aqueous extract was filtered and kept in refrigerator for further use.

2.3. Preparation of ZnO NPs using the aqueous extract of Euphorbia petiolata leaf

30 mL of plant extract was added to 50 mL zinc nitrate (1 M) dropwise under reflux condition at 80 °C for 2 hr until changing the color following the surface plasmin resonance effect (as monitored by Uv-vis technique) and formation of some white precipitation. The precipitation was completely separated using centrifugation at 7000 rpm and obtained powder washed with methanol and distilled water to remove possible contaminations. Finally annealing carried out in a muffle furnace at 400 °C for 2 h, Scheme 1. The obtained nanoparticles were identified using FT-IR, Uv-Vis, XRD, SEM and EDS techniques. Furthermore, the possible mechanism for the formation of green synthesized nanoparticles is presented, Scheme 2.



Scheme 1. Green synthesis of ZnO NPs using the extract of *Euphorbia petiolata*

2.4. Antimicrobial activity

The *Escherichia coli* bacterial strain was used during this study, Scheme 1. Disc diffusion method was carried out by using standard protocol, (Madan et al., 2016). The overnight bacterial culture (100 µL) introduced over Muller Hinton Agar plates with a sterile glass rod and minimum inhibition concentration (MIC) determined for each sample, Table 1. Also, the positive and negative controls were Chloramphenicol and prepared discs using sterile distilled water. Briefly, 100 µL of the aqueous plant extract and ZnO NPs solutions (with different concentrations) were loaded on provided discs (6 mm D) and allowed to dry before being placed on the agar. Each sample was tested to triplicate and the plates were inoculated at 37°C for 24 hours after incubation. Finally, the diameter of inhibition zones was measured and tabulated, Figure 8.

3. Results and Discussions

Through this study, the ZnO NPs were green synthesized using *Euphorbia petiolata* leaf extract without any stabilizer, surfactant and hazardous chemicals and its stability was further investigated since 2 hours to 20 days using Uv-vis spectroscopy. Furthermore, the antibacterial ability of the mentioned nanoparticles were studied against *E. coli* according disc diffusion method.

3.1. Spectroscopic results of the plant extract

Figure 2 depicted Uv-vis spectra of plant extract (a) and green synthesized ZnO NPs (b-e), respectively. For plant extract, both signals at 338 nm and 250 nm are for cinamoyl and benzoyl systems of flavonoids. Because of the application of Uv-vis signals as fingerprint and specification of flavonoids, these absorbent bonds revealed the presence of antioxidant flavonoids in plant extract, [31].

3.2. Identification of the green synthesized ZnO NPs

The UV-Vis spectrum of green synthesized ZnO NPs characterized the influence of surface plasmon resonance due to the significant changes in the absorbance maxima which indicates the interaction of phytochemicals with zinc ions within the plant extract and change the color of the reaction, (Scheme 2). Furthermore, the green nanoparticles show a suitable stability even after 2 weeks due to the adsorption of antioxidant phytochemicals on the nanosurface and protect it from decomposition and deformation processes, Figure 2.

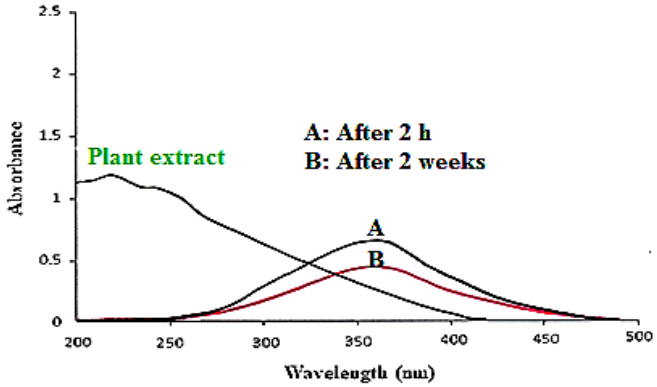
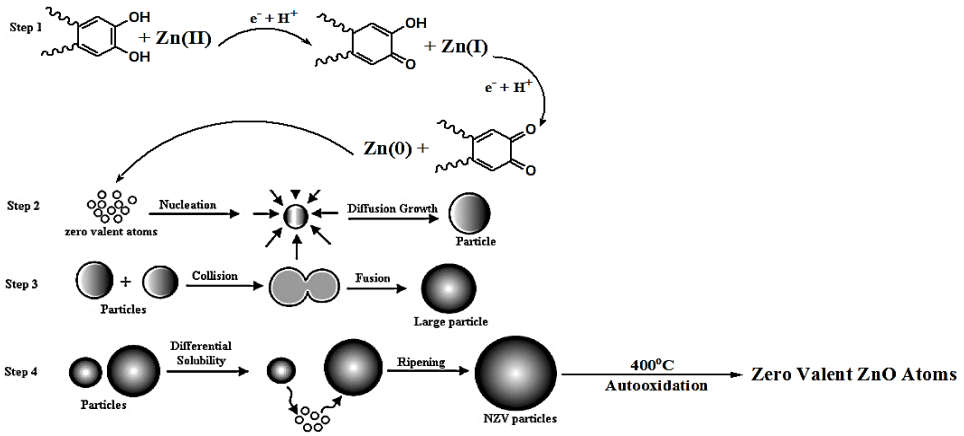


Figure 2. UV-Vis spectrum of *Euphorbia petiolata* extract (a) and green synthesized ZnO NPs at times

As scheme 1 shows, the formation of ZnO NPs as a white crystalline powder is for reducing performance of antioxidant phenolics of the plant extract. In fact after formation of Zn nanoparticles, for their large surface area they start to nucleation and more Zn ions adsorb on the nanosurface on which then convert to nano state undergo the plant

reducing potential. This process continues to increase the nano-layers and form a mass of Zn nanostructures through the ripening mechanism. Finally under annealing temperature at 400°C the mass of Zn nanoparticles oxidized and produce the crystalline ZnO NPs.



Scheme 2. Mechanism for green synthesis of crystalline ZnO NPs using the reducing ability of *Euphorbia petiolata* extract

Based on the Figure 3A, the FT-IR signals of the plant extract show main peaks at 3400, 1690, 1570 and 1200 cm-1 for OH, Carbonyl, C=C aromatic and C-O stretching bonds, respectively. The above functional groups are a strong support for the presence of antioxidant phenolic compounds in the plant extract. Also the FT-IR spectrum of the green synthesized ZnO NPs depicted the adsorption of phytochemicals on the surface of nanostructure as stabilizing and capping agents which strongly protect the nanoparticles from decomposition, deformation and coagulation processes. This fact is easily depicted in Figure 3B on which signals mainly at 3450, 1710 and 1530 cm-1 indicate the OH, Carbonyl and C-Sp2 of aromatics, respectively.

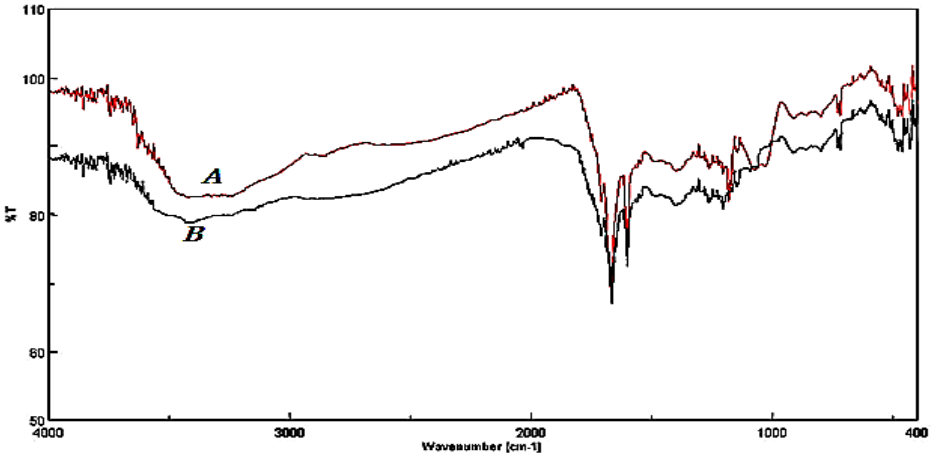


Figure3. The FT-IR spectrum of *Euphorbia petiolata* extract (A) and green synthesized ZnO NPs (B)

Figure 4 shows the X-ray diffraction (XRD) pattern of green synthesized ZnO nanopowder ranging 0 to 90 2theta degree. This pattern revealed the crystallinity of green synthesized nanostructures. The XRD signals at 31.841°, 34.507°, 36.324°, 47.592°, 56.634°, 62.895°, 66.426°, 67.983°, 69.091°, and 76.987° shows the (100), (002), (101), (102), (110), (103), (002), (112), (201), and (202) planes, respectively. The result of XRD pattern of ZnO NPs obviously shows its crystalline monoclinic structure based on the International Center of Diffraction Data card (JCPDS-36-1451). Furthermore the lack of other phase signals and their intensity indicate the phase purity of green

synthesized ZnO nanopowder. Finally the obtained XRD result of ZnO nanoparticles is in good agreement with those reported from the previous literatures [8-12].

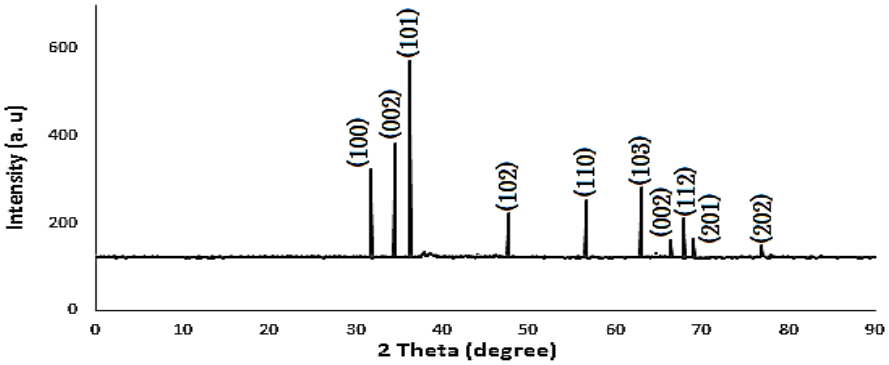


Figure4. XRD pattern of green synthesized ZnO NPs

In figure 5, the different SEM micrographs of the green synthesized ZnO NPs has been depicted to further study its morphology. As can be seen, the SEM image demonstrates the formation of the ZnO nanoparticles as relatively spongy shape nanoparticle as well as a number of aggregates.

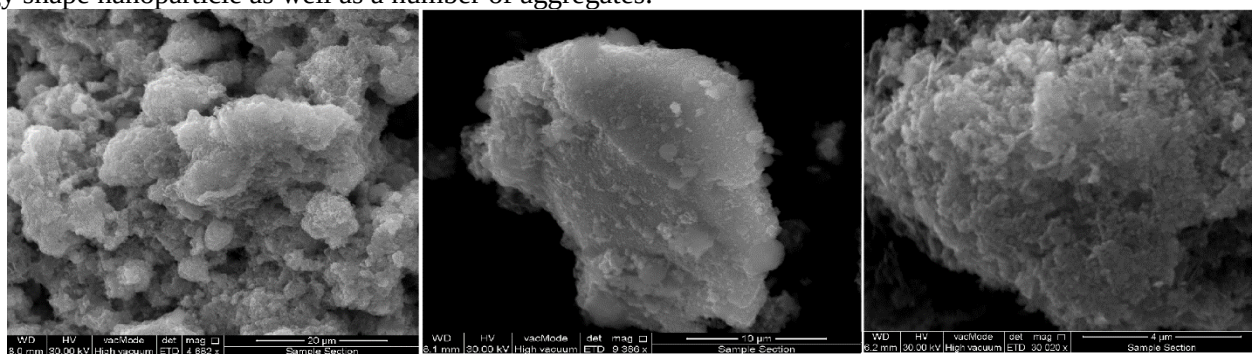


Figure 5. SEM micrographs of green synthesized ZnO NPs

Figure 6 shows the EDX spectrum of ZnO NPs synthesized using bioreduction method. The EDS analysis confirmed the presence of the zinc and oxygen. According to the EDS, XRD and SEM analysis, we can confirm that ZnO NPs were exactly prepared through green synthesized method. Moreover, the result obtained from EDS and XRD analysis support the purity of biosynthesized nanoparticles.

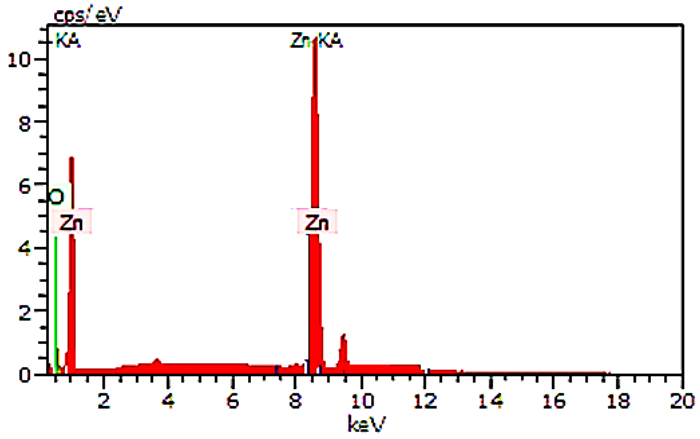


Figure 6. EDS analysis of green synthesized ZnO NPs using the extract of Euphorbia petiolata

3.3. Antibacterial activity of green synthesized ZnO NPs

The antibacterial activity of the plant extract and ZnO NPs was studied against *Escherichia coli* bacteria by disk diffusion method. The concentrations used for investigation of ZnO NPs were 1% (10 mg/ml), 5% (50 mg/ml), 10% (100 mg/ ml), 15% (150 mg/ml) and 20% (200 mg/ml) respectively. The results were compared with the chloramphenicol as a positive control. For reporting the result of the antibiogram test the minimum protection zone was reported for each test. According the figure 7 and Table 1(entry 2), the plant extract demonstrated no antibacterial activity against E. Coli in concentrations less than 15% but the green synthesized ZnO NPs showed the antibacterial activity in a good potential except for concentration of 1%, Table 1.

Table 1. the antibacterial activity of Green synthesized ZnO NPs and Euphorbia petiolata on *E.coli*

Entry	Compound	20 mg/ml	15% mg/ml	10% mg/ml	5% mg/ml	1% mg/ml
1	Green	N1:12mm	N1:11mm	N1:13mm	N1:10mm	N1:-
	Synthesized	N2:12mm	N2:14mm	N2:12mm	N2:10mm	N2:-
	ZnO NPs	N3:12mm	N3:13mm	N3:10mm	N3:9mm	N3:-
		C+:12mm	C+:12mm	C+:11mm	C+:12mm	
2	Aqueous	N1:11mm	N1:9mm	N1:-	N1:-	N1:-
	plant Extract	N2:9mm	N2:11mm	N2:-	N2:-	N2:-
		N3:9mm	N3:14mm	N3:-	N3:-	N3:-
		C+:10mm	C+:12mm			

[a]*C+; Chloramphenicol disc diameter (positive control), n; test frequency*

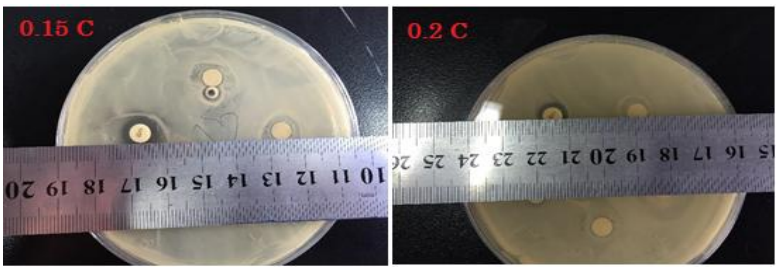


Figure 7. Antibacterial activity of plant aqueous extract against *E. Coli*

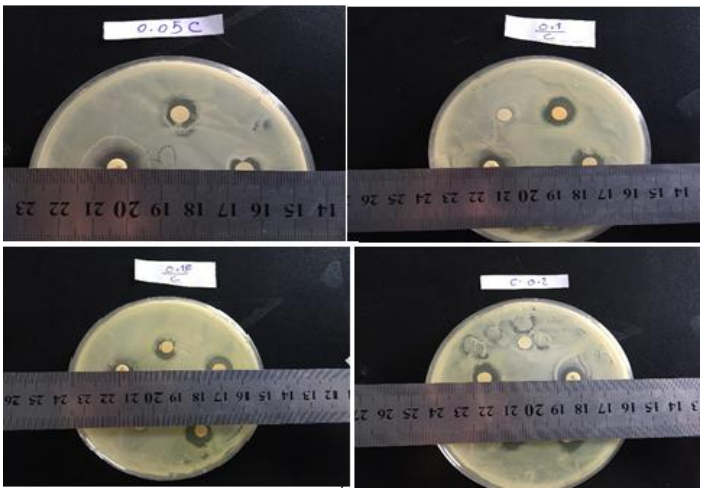


Figure 8. Antibacterial activity of green synthesized ZnO NPs against *E. Coli*

As it shown from Figure 8, in both concentrations of 15% and 20%, the biosynthesized NPs showed more antibacterial activity than the plant extract which probably refers to the accumulation of bioactive phytochemicals adsorbed on the extensive surface of nanoparticles as capping and stabilizing agents (as confirmed through FT-IR spectra). Therefore the study confirmed that the concentration of both compounds are an important factor concerning their antibacterial activity on which in concentration 15% both plant extract and ZnO NPs demonstrated a good antibacterial activity against E. Coli compared to positive control.

4. Conclusions

In summary, an efficient, green, biological, low-cost and sustainable protocol for the synthesis of the ZnO nanoparticles has been developed. The method involves the use of *Euphorbia petiolata* extract as reducing, capping and stabilizing agent. All techniques of Uv-vis, FT-IR, XRD, SEM and EDS strongly confirmed the biosynthesis and characteristic of nanoparticles. The significant advantages of this methodology are short reaction time, fast, single step, simple and eco-friendly synthesis of the nanoparticles, elimination of hazardous materials, extra surfactant or reductant, organic solvent and a simple work-up procedure. The antibacterial ability of green synthesized nanoparticles and plant extract against *E. Coli* was then evaluated compared to the Chloramphenicol as positive control. It was demonstrated that the obtained nanoparticles have considerable antibacterial ability against *E. Coli* compared to the plant extract and positive control.

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