Chemistry Research Journal, 2018, 3(5):53-59

Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Comparison of antimicrobial activity of bio-synthesized silver and zinc oxide nanopar-ticles using *Centaurea cyanus* L. (Cornflower) Flower extract

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Abstract In this study, the extract of *Centaurea cyanus L.* for production of silver and zinc oxide nanoparticle without use of any chemical agent was investigated. The silver (AgNPs) and zinc oxide nanoparticles (ZnONPs) showed strong antibacterial activity against both tested *Escherichia coli* O157:H7 (Gram negative) and *Staphylococcus aureus* (Gram positive) bacteria. The antibacterial activity of bio-synthesized nanoparticles against two pathogens was assessed by minimal inhibitory concentration (MIC) assays. The MIC values of AgNPs and ZnONPs of 10.63 µg/mL and 12.5 µg/mL for *E. coli* O157:H7 AgNPs and *Staphylococcus aureus* against ZnONPs wereat 3.32 µg/mL and 6.25 µg/mL for, respectively. Biologically synthesized nanoparticles were characterized by Scanning Electron Microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), UV-Vis spectroscopy, X-ray diffraction (XRD) and Zeta potential analysis. This study concludes that the bio- synthesized AgNPs and ZnONPs may be used as an effective antimicrobial activity, so it can be projected as future generation antimicrobial agents and designing newer drugs.

Keywords Biological synthesis, *Centaurea cyanus L.*, silver nanoparticle, zinc oxide nanoparticle, antimicrobial activity

1. Introduction

Nanotechnology and nanomaterials has gained increased attention in different field of technology [1]. The metallic nanoparticles that have large surface area so; they can be used antibacterial agent [2].

Nanoparticles can be synthesized by different physical, chemical and biological methods [3]. The risk of toxic compounds used chemical and physical methods limits the biomedical applications. So, the using of biological materials ie plants, plant components, bacteria for synthesis are safe and eco-friendly [4]. Plant based synthesis has some advantages such as it is faster and stable, getting different size and shape of NPs in comparably those obtained by microorganisms. Many investigations showed silver and zinc oxide nanoparticles are exhibit antibacterial, antioxidant and photocatalytic properties [5-6]. Many studies showed that silver and zinc oxide nanoparticles are superior product from the field of nanotechnology because of their exclusive properties such as the most important antibacterial, anti-viral, antifungal and anti-inflammatory activities and good stability [7-8]. For these properties, silver and zinc oxide nanoparticles have been used most widely in the different industries *i.e.* health, food packaging, textile and other applications.

In this study, easy and biological synthesis of silver and zinc oxide nanoparticles by an environmentally friendly procedure was investigated. For this purpose, *Centaurea cyanus* L. plant powders were used for the bio-synthesis of silver and zinc oxide nanoparticles. Additionally, the evaluation of their antibacterial activity against *E. coli* and *S. aureus* which are known as human pathogenic bacteria was investigated.

2. Materials and methods

2.1. Bio-synthesis of silver and zinc oxide nanoparticles

For the bio-synthesis of silver and zinc oxide nanoparticles, a fresh *Centaurea cyanus* L. plant was used in this study. Briefly, 10 g dried plant was added into 100 ml boiling deionized water for 30 min. The obtained extract was left to cool at room temperature, and then filtered using Whatman No. 1 filter paper. The filtrate was stored at 4 °C until being used in the green synthesis of AgNPs and ZnONPs.

2.2. Bio-synthesis of silver nanoparticles using *Centaurea cyanus* L. extract

A specific procedure was employed, where 10 ml of *Centaurea cyanus* L. aqueous extract was taken from the stock solution and 3 mM of $AgNO_3$ was dissolved in the *Centaurea cyanus* L. extract solution using magnetic stirrer. After that, the solution was boiled at 60–80 °C. The color change of mixture indicated the complete of nanoparticle synthesis which was validated by absorbance peak by UV–Vis spectroscopy.

2.3. Bio-synthesis of zinc oxide nanoparticles using *Centaurea cyanus* L. extract

A specific procedure was employed, where 10 ml of *Centaurea cyanus* L. aqueous extract was taken from the stock solution and 2 g of zinc nitrate hexahydrate crystal was dissolved in the *Centaurea cyanus* L. extract solution using magnetic stirrer. After that, the solution was boiled at 60-80 °C. The color change of mixture indicated the complete of nanoparticle synthesis which was validated by absorbance peak by UV–Vis spectroscopy.

2.4. Characterization of bio-synthesized silver and zinc oxide nanoparticles

Characterization of AgNPs and ZnONPs were made using by a dynamic light scattering (DLS), UV-Vis spectrometry, scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FT-IR). Crystalline metallic silver and zinc was examined by X-ray diffractometer.

A few drops of the concentrated AgNPs and ZnONPs solution were deposited on a carbon tape covered stub, and left overnight for drying. Then, the stub was coated with gold using a sputter coater to produce clear images from the SEM (ZEISS EVO LS10). The AgNPs and ZnONPs were well dispersed in water and the solution was used to determine the absorbance of the AgNPs and ZnONPs. The well dispersed AgNPs and ZnONPs solution was also used for measuring the effective diameter of the AgNPs and ZnONPs with a DLS. The formation of the AgNPs and ZnONPs and existence of the plant extract on the surface of the AgNPs and ZnONPs were both proven by FT-IR. For FT-IR analysis, the AgNPs and ZnONPs solution was centrifuged at 10.000 rpm for 15 min and the precipitated AgNPs and ZnONPs were allowed to dry at 60 °C.

2.5. Microorganisms and growth conditions

In this study, microorganisms (*E. coli* O157:H7 NCTC 12900, *S. aureus* ATCC 29213) were obtained from the culture collections of Department of Food Hygiene and Technologies, Faculty of Veterinary, Erciyes University, Kayseri, Turkey. Microorganisms were plated on blood agar (Oxoid, CM0271) and incubated at 37 °C for 18-24 h. After incubation, 2-3 colonies of each organism taken from blood agar were inoculated to 5 ml Mueller Hinton broth (Oxoid, CM0405) and incubated overnight at 37 °C. After that the suspension adjusted to 0.5 McFarland turbidity (approximately 10^8 cfu ml^{-1} for bacteria).

2.6. Determination of antibacterial activity with micro dilution broth method

The biologically synthesized silver and zinc oxide nanoparticles were tested against pathogenic bacterial strains such as Gram (-) *E.coli* O157:H7 (NCTC 12900) and Gram (+) *S. aureus* (ATCC 29213). To determine the antibacterial activity and the minimum inhibitory concentrations (MICs) of microorganisms studied, the well broth micro dilution method was used with 96-well plates [9]. The biologically synthesized silver and zinc oxide nanoparticles were diluted6 serial two-fold in Muller-Hinton broth (Oxoid, CM0405) in a 96-well microtiter plates. Afterwards, 100 µl of freshly grown bacteria standardized until a bacterial number of 1×10^8 CFU/ml in Muller-Hinton broth was

reached, was added to each well and.The micro dilution test comprised positive control without extract and negative control lacking microorganisms under the same conditions. Plates were incubated aerobically at 37 °C for 24 h. To determine antimicrobial activity and MICs, 10ul broth was spot inoculated onto Mueller-Hinton agar (Oxoid CM0337) and incubated at 37 °C for 24 hours. After the incubation, the inhibition of bacterial growth was recorded and interpreted as the MIC [9]. The antibacterial activities of the NPs were compared with commonly used antibiotics including ciprofloxacin and vancomycin as positive control. Tests were performed in duplicate.

3. Results and Discussion

3.1. Characterization of AgNPs and ZnONPs suspensions

AgNPs and ZnONPs synthesis was found to be successful; primary indication was change in yellow colour of extract to dark grey mixing with $AgNO_3$ and $Zn(NO_3)_2.6H_2O$ solution. Figure 1 A and B show that the size of the AgNPs is around 30 nm and the size of the ZnONPs is around 25nm. Small aggregations were observed in the SEM image. The small aggregations of the AgNPs may increase the dynamic size.

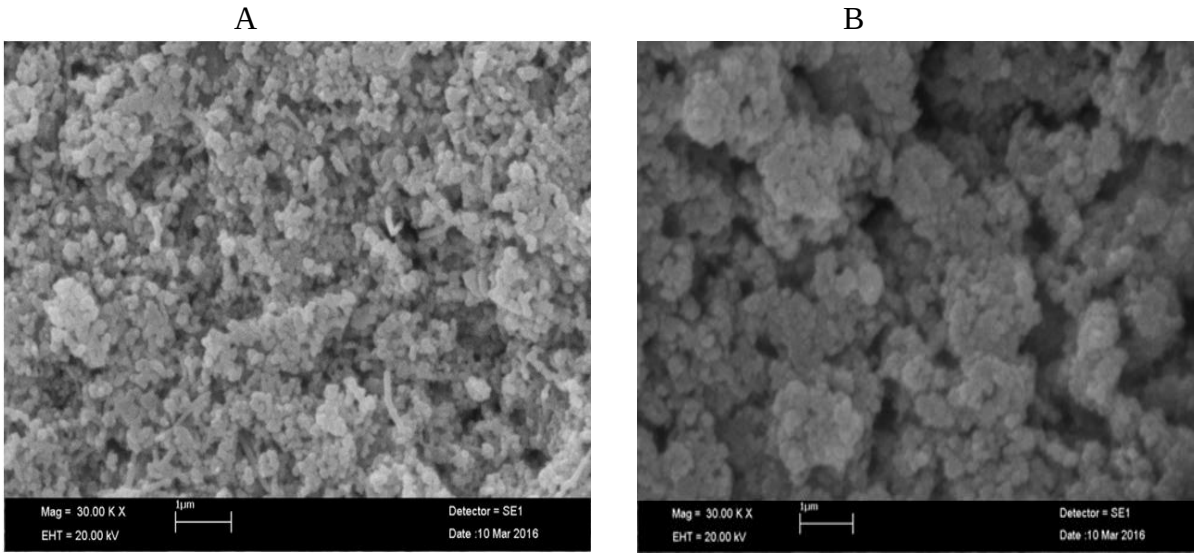

Figure 1: An image of A: silver nanoparticles (AgNP), B: zinc oxide nanoparticle (ZnONP) in the Centaurea cyanus L. culture medium

Nano-particulate silver showed a well-defined absorption peak in visible region at 273 and 454 nm (Fig. 2A). The interaction of AgNPs with extract of *Centaurea cyanus* L. validated the reduction of Ag^+ ions to Ag^0 by the reactive groups that may get in turn oxidized to other species. Similarly, nanoZnONPs show two absorbance peaks at 270 nm and 323 nm, which correspond to the presence of the plant extract on the ZnONPs surfaces and ZnONPs, respectively (Fig.2B).

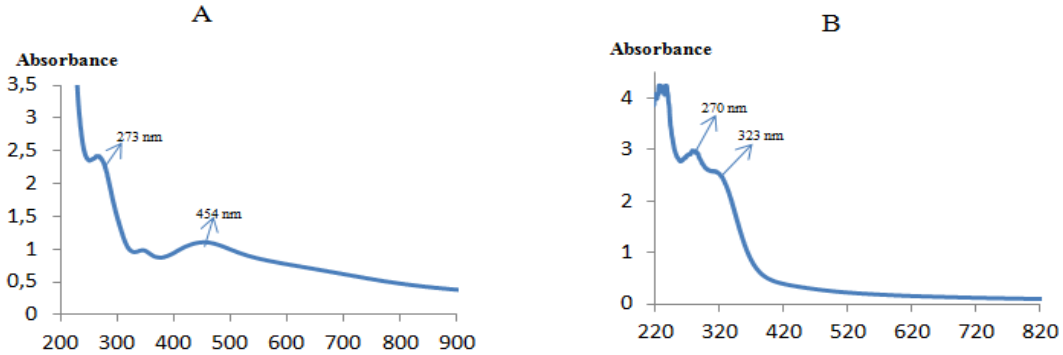

Figure 2: An image of UV-Vis results of A: silver nanoparticles (AgNP), B: zinc oxide nanoparticle (ZnONP) in the Centaurea cyanus L. culture medium

The UV–Vis spectroscopy is generally used in different studies to examine the size and shape of nanoparticles in aqueous suspension. Sastry *et al*., (1998) stated that the optical absorption spectrum of metal nanoparticles is dominated by surface plasmon resonance (SPR) [10].

The formation of the AgNPs and ZnONPs and the presence of the plant extract as capping agents on the AgNPs and ZnONPs surfaces were evaluated by FT-IR (Perkin Elmer Spectrum 400, Fig.3 A and B). The alcohol (O-H) stretching peaks was observed at ~3669 cm^{-1} and ~3202 cm^{-1}, respectively. The stretching bands at ~2972 and 2951 cm^{-1} were related to the alkane (C-H). The bands region at 2909–3698 $cm^{-1}$and 942-1714 cm^{-1} related to bioactive compounds/aromatic groups [11]. The aromatic stretching (C=C) emerged at ~1382 and 1450 cm^{-1}, and the stretching bands at ~1248 and 1375 cm^{-1} were related to the alkyl halide (C-F). The alcohol (C-O) stretching vibrations were observed at ~1066 and 1050 cm^{-1}. The functional groups from plant tissues can interact with different kind of metal salts and this process determine to nanoparticles formation [12]. In this study, FT-IR results recognized the possible biomolecules are responsible for the reduction and stabilization of AgNPs and ZnONPs synthesized.

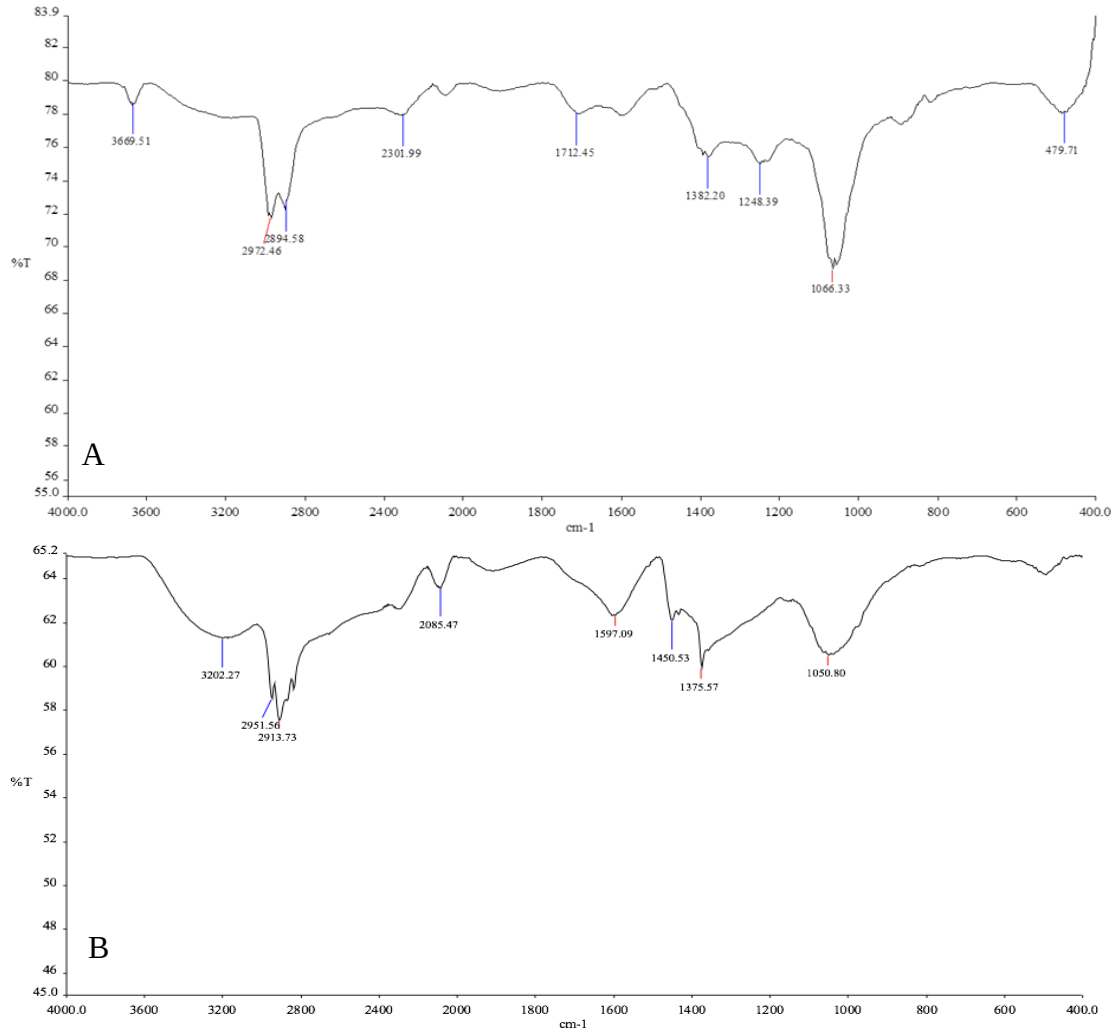

Figure 3: FTIR spectrums of A: silver nanoparticles (AgNP), B: zinc oxide nanoparticle (ZnONP) in the Centaurea cyanus L. culture medium. (T= Transmittance; cm^{-1}= Wavelength.nm).

X-ray Diffraction technique (BRUKER AXS D8) was used for characterization of the structures of AgNPs and ZnONPs. The AgNPs and ZnONPs precipitate was dried at 80 °C before XRD analysis and nanoparticles were scanned under the range of scattering angle (2θ) between 10 ^{o} to 90 ^{o}. The diffraction peaks appeared on 38.2 ^{o}, 44.3 ^{o} and 64.6 ^{o} (respectively, correspond to the (1 1 1), (2 0 0) and (2 2 0) planes) refers to face centered cubic lattice structure of AgNPs (Fig.4A). The diffraction peaks at 31.7^{o} (1 0 0), 34.4^{o} (0 0 2), 36.2^{o} (1 0 1), 47.5^{o} (1 0 2), 56.5^{o} (1 1 0), 62.8^{o} (1 0 3), 67.9^{o} (1 1 2) and 69.0^{o} (2 0 1) related to crystal planes of hexagonal structure of ZnONPs (Fig.4B).

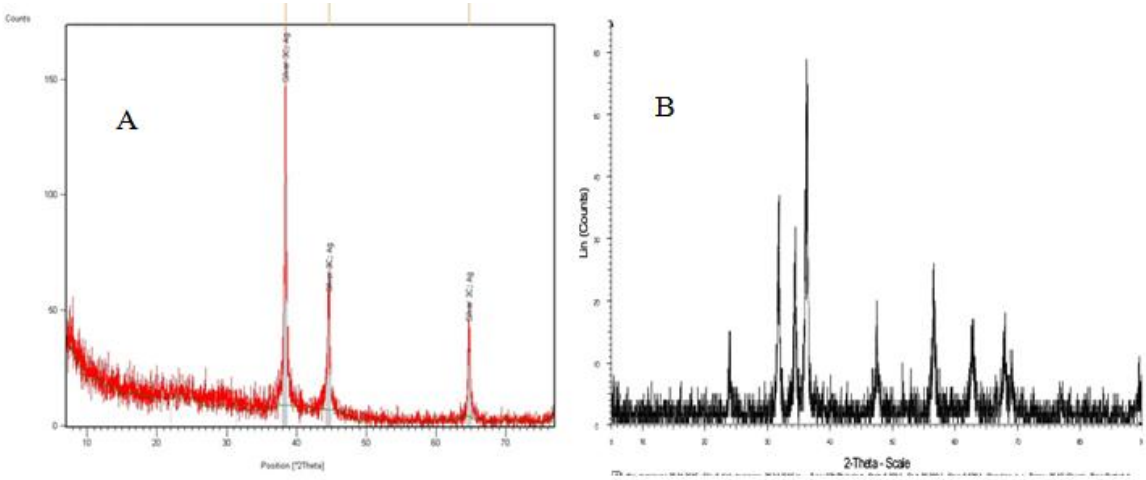

Figure 4: XRD patterns of AgNP (A) and ZnONP (B)

Zeta potential was taken as the mean value of different measurements. Zeta potential on the surface of AgNPs and ZnONPs was found to be −22.5 mV and – 22 mV, respectively, thereby this can be anticipated that AgNPs and ZnONPs showed good stability in water due to the electrostatic repulsive forces (Fig. 5 A and B). This stability and zeta potential were clues for an electrostatic mechanism due to adsorption of secondary metabolites. The obtained zeta potential value for the bio-syntesized AgNPs and ZnONPs prove that they are good stable. These results are agreed with obtained by Kitler *et al*., (2010) [13].

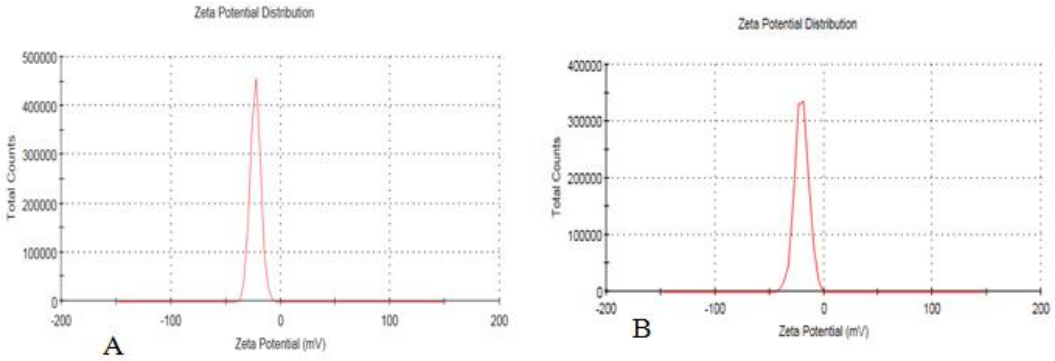

Figure 5: An image of Zeta Potential for A: AgNPs and B: ZnONPs

3.2. Antimicrobial assay of AgNPs and ZnONPs

The silver and zinc oxide nanoparticles demonstrated strong antibacterial activity against both tested bacterial strains in the MIC assay (Table 1). However, AgNPs broadly presented a faintly higher efficacy than ZnONPs. In detail, the MIC values of ZnONPs and AgNPs detected as values of 12.5 µg/ml and 10.63 µg/ml against *S. aureus*, respectively. Also, 6.25 µg and 3.32 µg concentrations per ml were sufficient for killing of *E.coli* O157:H7 with ZnONPs and AgNPs, respectively.

Similar to present study, Bhumi and Savithramma (2014) reported that the ZnO-NPs had antimicrobial effect on *E. coli* and *S. aureus* [14]. Navale *et al*. (2015) detected that the MIC value of ZnO-NPs against *S. aureus* was 40µg/ml

[15]. According to study conducted by Emami-Karvani et al (2011), ZnO nanoparticles have antibacterial effect at the 10 mg/ml concentrations against *E. coli* [16]. Similar observations reported by Mostafa et al., (2015) and Lkhagvajav *et al*. (2011), they suggested that the MIC of AgNPs against *S. aureus* and *E. coli* were 2.5 and 2 µg/mL, respectively [17, 18]. However, Fayaz *et al.*, (2010) showed that AgNPs were effective to Gram negative bacteria at 30–35 µg/mL versus were effective against Gram positive bacteria at 65–80 µg/mL concentrations [19]. Shameli *et al.*, (2012) recorded that AgNPs were effective against *S. aureus* and *Salmonella typhi* at 20 µL of AgNPs [20].

Table 1: Minimum inhibitory concentrations of bio-synthesized nano/silver and zinc oxide against *E. coli* and *S. Aureus*

Names of the tested bacteria	MIC Values Control antibiotics (µg/ml)								ZnONP	AgNP
	Vancomycin				Ciprofloxacin				MIC value (µg/ml)	MIC value (µg/ml)
	S*	I*			S*	I*	R*	T		
S. aureus	≤2	4-8	≥16	1	≤1	2	≥4	1	12.5	10.6
E. coli	-	-	-	-	≤1	2	≥4	1	6.25	3.32

Different investigations showed that the sizes of zinc nanoparticles are important for antibacterial affectivity [21]. Results from this study indicated that the biological synthesized ZnONPs have improved antimicrobial activity. The antibacterial activity of ZnONPs was more effective than ciprofloxacin and vancomycin (these are commercial antibiotics). So, AgNPs and ZnONPs may be used as a substitute to commercial antibiotics. Our results are supported by the observations obtained by different authors [21, 22]. According to studies, the differences in bacteria's cell membrane structure can cause the different nanoparticle toxicity.

Different studies showed that after contact with the bacterial membrane, nanoparticles generates high rate of reactive oxygen species. So, the death of bacteria due to chemical interactions between hydrogen peroxide and membrane proteins [23, 24].

4. Conclusions

In this study, AgNPs and ZnONPs green synthesized by using *Centaurea cyanus* L. extract were tested for their antibacterial. AgNPs and ZnONPs formation was justified by UV-Vis; FTIR spectrum; SEM and XRD. The bio-synthesized AgNPs and ZnONPs have showed good antibacterial activity against pathogenic bacteria if compared to the antimicrobics currently marketed. Thus, we concluded that the present green synthesis route may be considered further to produce antimicrobial agent useful in a wide array of biomedical and pharmaceutical applications.

Conflict of Interest

The authors declare that they have no conflict of interest.

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