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Research Article

Silver Nanoparticles Obtained by Aqueous or Ethanolic *Aloe vera* Extracts: An Assessment of the Antibacterial Activity and Mercury Removal Capability

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Silver nanoparticles (AgNPs) were synthesized by chemical reduction of Ag^+ ions (from silver nitrate $AgNO_3$), using aqueous or ethanolic *Aloe vera* extracts as reducing, stabilizing, and size control agent. The nanoparticles' sizes were between 2 and 7 nm for ethanolic extract and between 3 and 14 nm for aqueous extract, as measured by High-Resolution Transmission Electron Microscope (HRTEM). The antibacterial activity against a mesophilic microorganism, *Kocuria varians*, a Gram-positive coccus, was measured by counting bacterial colonies in agar plate for both extracts. We found that 4% effective concentration is the lowest concentration that completely inhibited visible growth. Mercury removal was investigated by Atomic Absorption Spectroscopy (AAS) measurements, where it was shown that it is not necessary to use high concentrations of nanoparticles for effective removal of mercury inasmuch as with a 20% V/V concentration of both extracts; the Hg(II) removal percentage was above 95%. These results show that the mercury remaining unremoved from the different essays is below the level allowed by World Health Organization (WHO) and the Environmental Protection Agency (EPA).

1. Introduction

The synthesis of nanomaterials is currently one of the most active areas in nanoscience. Special attention has been dedicated to nanomaterials that help improve the human quality life. A remarkable example is the silver nanoparticles (AgNPs) which are known by their inhibitory and bactericidal effects.

AgNPs can be produced with various sizes and shapes depending on the fabrication method which can be physical, chemical, biological, and hybrid. The chemical methods use toxic chemicals, which are not friendly to environment, making them unsuitable for biomedical applications. Specifically, the widely used chemical reduction methods [1] usually employ toxic and perilous chemicals that are responsible for various biological risks. On the other hand, physical methods are expensive and incompatible with sizeable production of nanoparticles. Therefore, to avoid toxic and hazardous chemicals, the green synthesis methods have been developed, attracting significant interest because they are environment-friendly, rapid, facile, and energy-efficient [2]. Green synthesis using huge biological molecules derived from plant extracts [3] could facilitate size and morphology control of metal nanoparticles due to the presence of an innumerable quantity of biomolecules possessing bioreduction and biostabilization ability [4]. Specifically, many plants have been used for silver nanoparticles synthesis [3, 5], such as stem bark of *Callicarpa maingayi* [6], *Terminalia cuneata* [7], *Illicium verum* (star anise) [8], and pod extract of *Acacia nilotica* [9].

Aloe vera extracts have been used for the synthesis of stable AgNPs in several previous articles investigating their antibacterial, antifungal, and mosquitocidal activity [10–13]. *Aloe vera* extracts have substances that lead to steric repulsion between individuals preventing nanoparticles from aggregation [14]. Using *Aloe vera* as surfactant prevents nuclei aggregation by decreasing the total surface energy because it contains a multitude of chemical constituents such as amino acids, enzymes, minerals, vitamins (A, C, and E), anthraquinones, lignin, monosaccharide, polysaccharides, salicylic acid, saponins, sterols, and minerals (calcium, phosphorous, potassium, iron, sodium, magnesium, manganese, copper, chromium, and zinc) [15].

The antibacterial properties of silver have been known since ancient times. It has been used in water and air purification, biomedical application, catalytic activity, household products, cosmetics [18–20], food production [21], and clothing [22, 23]. Due to its small size and large surface to volume ratios, AgNPs might exhibit additional antimicrobial capabilities not exerted by ionic silver. Compared with their bulk counterparts, silver nanoparticles have both chemical and physical differences in their properties [24].

AgNPs have also been used in the control of blood sugar levels [25], destruction of pesticides [26], and the removal of heavy metal ions such as Hg(II), Hg(I), Pb(II), and Cd(II) from water [27, 28]. Besides, Katok et al. have reported [29] that as the diameter of AgNPs is reduced below 32 nm, mercury(II) is reduced from water onto AgNPs. Esmaielzadeh Kandjani et al. have proposed ZnO/Ag nanoarrays, another nanostructured system, to remove Hg (II) due to its high selectivity because of the unique way in which mercury interacts with Ag nanoparticles [30].

Because mercury is one of the most toxic heavy metals and concerns the threats to environment and human health, other nanostructured systems have been proposed.

This study aims to evaluate the antibacterial activity and the mercury removal capability of AgNPs, which were synthesized by chemical reduction method of silver nitrate (AgNO3), using aqueous or ethanolic *Aloe vera* extracts as reducing, stabilizing, and size control agent. In a previous work, we reported synthesis of silver nanoparticles using a mixture of polyvinylpyrrolidone (PVP)-*Aloe vera*, as reducing and stabilizing agent, in order to control the particle size [31]. The morphology and particle size distribution were characterized by Transmission Electron Microscope (TEM); the antibacterial activity against mesophilic microorganism, *Kocuria varians*, a Gram-positive coccus, was measured by counting bacterial colonies in agar plate, while mercury removal was investigated by Atomic Absorption Spectroscopy (AAS) measurements.

2. Materials and Methods

2.1. Materials. Analytical grade silver nitrate ($AgNO_3$), ethanol absolute, sodium hydroxide (NaOH), sodium borohydride ($NaBH_4$), and hydrochloric acid (HCl) were purchased from Merck KGaA and mercury standard solution (Panreac) Hg = 1.000 ± 0.002 mg/L. The reagents were used as received.

2.2. Aloe vera Extracts Preparation. 15 g of inner leaf juice of *Aloe vera* leaves was heated at 80°C for 1 hour and then dried. It was used for both aqueous and ethanolic extracts, using a ratio of 0.1 : 3, dry material to solvent. The resulting extracts were used in all synthesis after being filtered by gravity.

2.3. Preparation of Silver Nanoparticles (AgNPs). The AgNPs were prepared by chemical reduction of an aqueous solution, 12 mM of $AgNO_3$. 50 mL of this solution is added to 30 mL of either aqueous or ethanolic *Aloe vera* extract. The whole reaction was carried out in presence of air and constant and neutral pH. The mixture was vigorously stirred at temperature of 57°C during 3 h and then heated 2°C/min to reach 80°C holding for 2 hours until obtaining a translucent solution with small suspended particles that could be removed by simple filtration (0.45 μm).

2.4. TEM Measurements. The particle size and morphology of silver nanoparticles were observed by High-Resolution Transmission Electron Microscopy (HRTEM) using a Tecnai F20 Super Twin TMP with field emission source, with resolution of 0.1 nm at 200 kV and 1.0 maximum magnification TEM MX camera GATAN US 1000XP-P. Samples for TEM measurements were suspended in ethanol and ultrasonically dispersed.

In this study, approximately 483 particles for ethanolic extract (or 93 particles for aqueous extract) were measured from several images using image analysis software (ImageJ) [32]. The histogram obtained was fitted using a Gauss distribution function.

2.5. Antibacterial Activity of Synthesized AgNPs. Initially, the inoculum was prepared using fresh rumen. Thereafter, the nutrient solution was prepared according to Siegert and Banks [33], obtaining a solution with a rumen concentration of 1 g/L. It was cultured with 10 mL of the inoculated solution and stirred for 24 hours. Nutrient broth solution according to the manufacturer's recommendation (8 g/L) was prepared and spread onto agar plate and incubated at 37°C for 6 h. After incubation, the content of microorganisms was evaluated according to the McFarland scale (1.7×10^8 CFU/mL). The specific bacteria in this solution were identified by microbiological analysis: *Kocuria varians*, a Gram-positive coccus. This microorganism found in the microbiota skin and mucosae of man and some mammals is responsible for different types of human infection [34, 35], brain abscess [36], and urinary infections [37].

To examine the bactericidal activity of AgNPs against *Kocuria varians*, the agar dilution method was used. It involves the incorporation of varying desired concentrations of the antimicrobial agent into an agar medium, habitually using serial twofold dilutions, followed by the inoculation of a defined microbial inoculum onto the agar plate surface. As nutrient medium, an agar plate count solution was used, which was sterilized at 20 pounds of pressure for 30 minutes in autoclave GEMMYCO SA 232 mark.

In a series of previously sterilized Petri dishes with agar, a solution of AgNPs was added so that the final nanoparticle concentration was 10%. After agar solidification, 0.1 mL of the

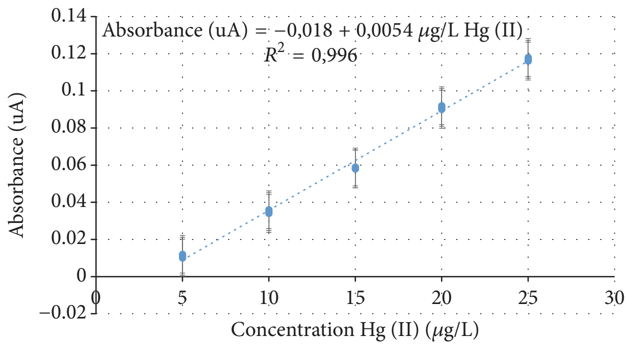


Figure 1: Calibration curve to determinate the quantity of Hg(II), which is a plot of the concentrations of standard Hg(II) (μg/L) versus the absorbance of Hg(II) at 253.7 nm.

inoculum was added and the plates were incubated at 37°C. After incubation, the number of colonies grown on the agar was counted [17]; all this procedure was done in triplicate. It was considered a positive control, which consisted of agar without nanoparticles and inoculated and a negative control which consisted of agar with nanoparticles but not inoculated.

2.6. Mercury Removal Capacity of Synthesized AgNPs. In order to evaluate the mercury removal capability of synthesized nanoparticles, a series of fixed volume dilutions of AgNPs (20, 40, 60, 80, and 100% V/V) were incorporated in an agar plate count matrix. When each system has been solidified, 2.5 mL standard solution of mercury, Hg(II), at different concentrations (5, 10, and 15 mg/L) was added in triplicate and kept in contact for 24 h. The quantification of Hg(II) in the solution was carried out by Atomic Absorption Spectrometer (Thermo Scientific™ CE 3000), fitted with a Thermo Scientific VP100; the steam system uses a solution of sodium borohydride ($NaBH_4$) and hydrochloric acid (HCl) to get the complex with the mercury.

The resulting calibration curve (Figure 1) shows that absorbance versus concentration of Hg(II) is linear over the 5 to 25 μg/L range and is suitable for quantitative work.

3. Results and Discussion

Figures 2(a) and 2(b) show TEM micrographs and corresponding size distribution histogram of AgNPs obtained using aqueous and ethanolic *Aloe vera* extracts, respectively. The TEM image confirms the presence of nanoparticles between 2 and 7 nm in size for ethanolic extract and between 3 and 14 nm for aqueous extract. Thus, AgNPs obtained by chemical reduction using ethanolic *Aloe vera* extract as a surfactant exhibited smaller sizes compared to AgNPs obtained from aqueous *Aloe vera* extract.

In previous works, stable AgNPs have been synthesized using aqueous *Aloe vera* extract. Reference [13] found that the AgNPs could be stable for at least three months and SEM analysis revealed that the AgNPs are predominantly spherical with average size of 25 nm. Other authors ([11, 12]) have used also SEM analysis, [12], showing that silver nanoparticles are cubical, rectangular, triangular, and spherical in shape with uniform distribution. The measured sizes of the agglomerated nanoparticles were in the range of 287.5–293.2 nm; however, the average size of an individual particle is estimated to be 70 nm. On the other hand, [11] showed almost spherical and cubic structures with a size range of 35–55 nm. Moreover, Chandran et al. [10] synthesized silver nanoparticles with average size of 15.2 ± 4.2 nm as estimated by TEM. Their analysis reveals that the silver nanoparticles are predominantly spherical; nonetheless the reaction proceeded only in the presence of ammonia, which facilitates the formation of a soluble silver complex (diammine silver(I) chloride) that then aids in the reduction.

We find similar results in terms of particle size, demonstrating the effectiveness of *Aloe vera* extracts as a reducing agent to mediate the green synthesis of silver nanoparticles; additionally, the good dispersibility mainly in ethanolic *Aloe vera* extract could be explained by a layer of organic material surrounding the synthesized AgNPs, as is shown in TEM images.

The resultant histograms show slight dispersion of the particle size of synthesized AgNPs for both ethanolic and aqueous extract distributions but confirm the presence of nanoparticles with size less than 15 nm for both extracts, as well as the presence of nanoparticles of greater size but in minimum quantities. These larger diameter AgNPs were vastly outnumbered by those with smaller diameters in the HRTEM images analyzed. Using ethanolic *Aloe vera* extract, most particles (~90.5%) are within a range size between 2 and 6 nm, while ~76.3% of those using aqueous *Aloe vera* extract are in this range size. This can be explained by the higher solubility power of ethanol for organic compounds yielding a lower particle size and less polydispersity than water.

The hexagonal patterns observed by electron diffraction (Figure 3) represent a proof of the crystalline nature of the prepared nanoparticles. The hexagonal symmetry of diffraction spots pattern (Scherrer ring pattern) shown in both cases confirmed that the spherical particles are well crystalline, and its face is indexed to (111) planes. The measured lattice spacing (~0.235 nm for both samples), corresponding to (111) planes of silver, is in agreement with previous reports [16]. The enlarged image of the Fast Fourier Transforms of High-Resolution Transmission Electron Microscopy, FFT pattern, shows additional diffraction spots that are probably associated with compounds of *Aloe vera* and ethanolic extracts. The values obtained are summarized in Table 1.

These results are in agreement with the measurement extracted directly from the enlarged micrograph of one particle. The lattice spacings of 0.265 nm and 0.237 nm, obtained for the aqueous and ethanolic *Aloe vera* extracts, respectively, were calculated from TEM images (Figure 3) using the ImageJ software [32]. Fringe periods represent metallic fcc Ag phase [38] and correspond to (111) planes of silver.

(i) Antibacterial Activity. The antibacterial activity of AgNPs obtained with both extracts against *Kocuria varians* was evaluated in triplicate using the agar dilution method, which involves the incorporation of varying desired concentrations of the antimicrobial agent (20, 40, 60, 80, and 100 (% V/V)

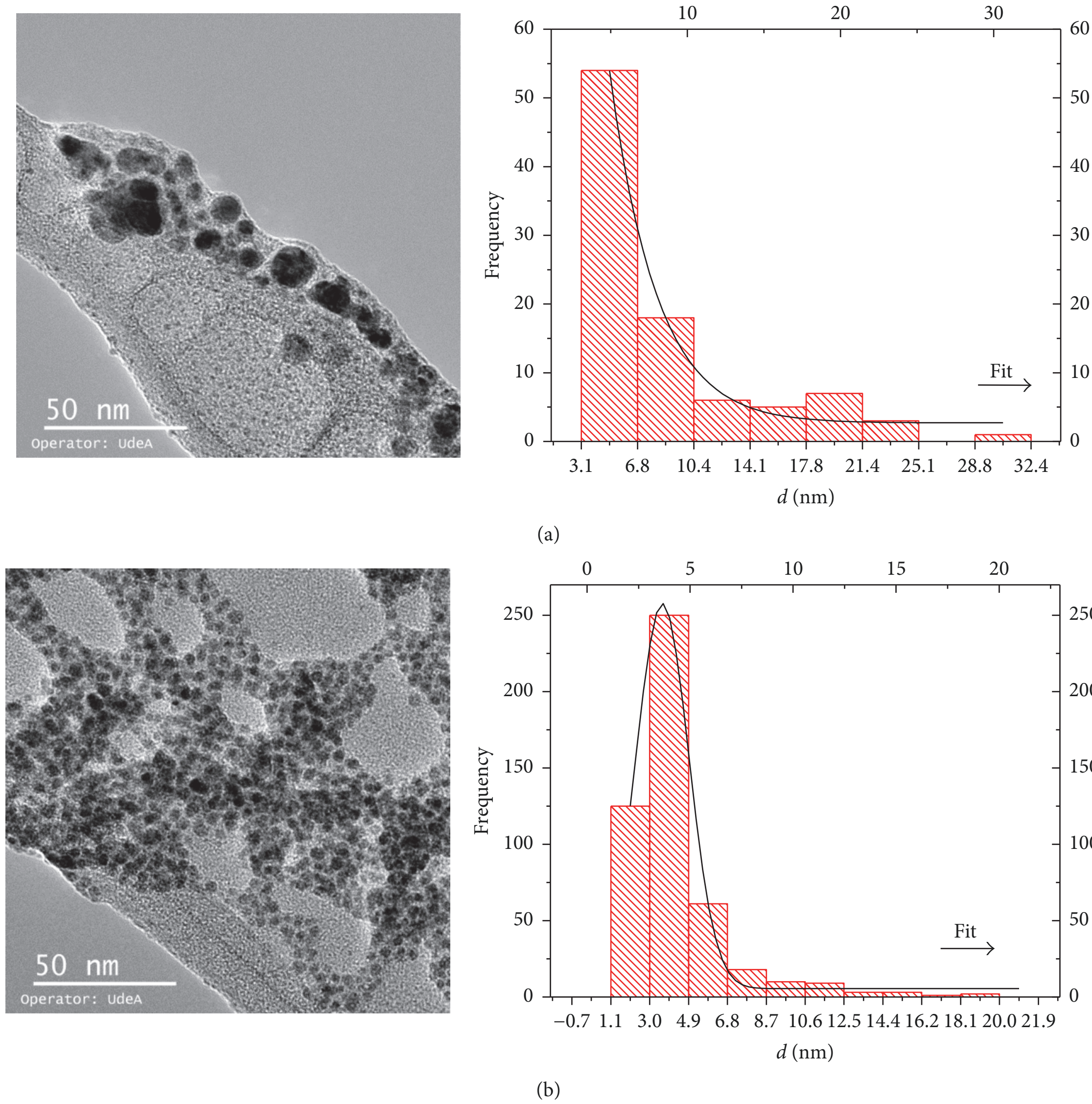


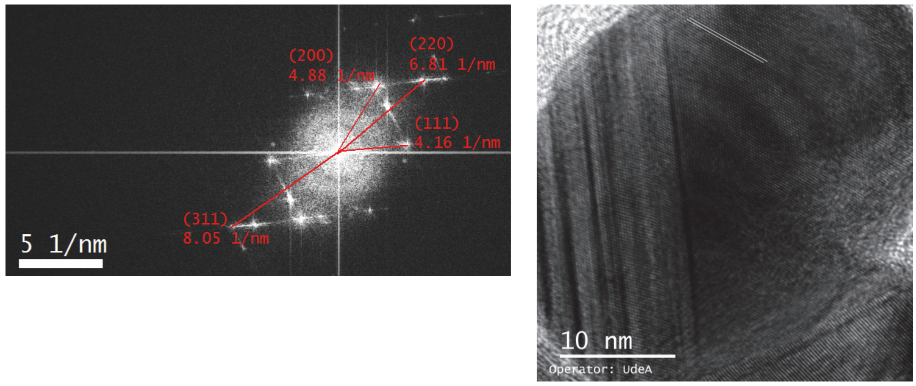
Figure 2: TEM micrographs of silver nanoparticles obtained from aqueous (a) or ethanolic (b) *Aloe vera* extracts.

Table 1: Planes distances of values found in this work compared with those reported in [16].

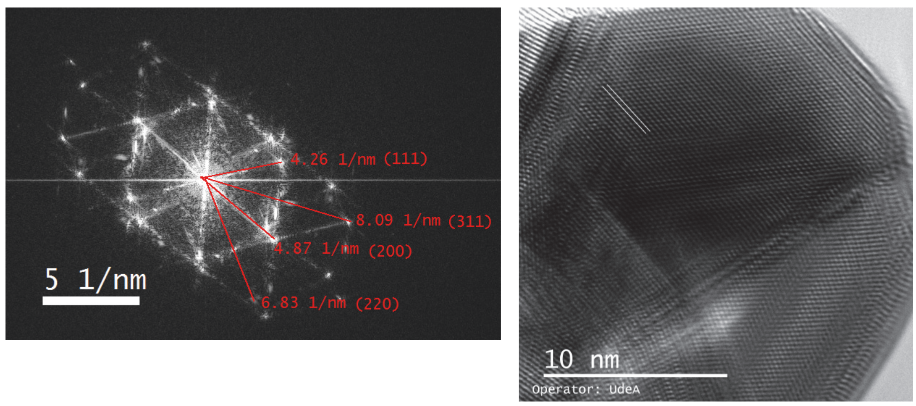
2θ (grad)[17]	d (nm)[16]	$1/d$ (1/nm)[16]	Miller index	TEM ($1/d$)(Figure 3(a))	TEM ($1/d$)(Figure 3(b))
38.3182	0.235	4.25	(111)	4.16	4.26
44.4975	0.203	4.92	(200)	4.88	4.87
64.6119	0.144	6.94	(220)	6.81	6.83
77.5385	0.123	8.13	(311)	8.05	8.09

of solution AgNPs) into an agar medium, followed by the inoculation of a defined microbial inoculum onto the agar plate surface. Bactericidal activity was shown at higher concentration (40, 60, 80, and 100%), while at 20% dilution, there was no antibacterial activity. The lowest concentration that completely inhibited visible growth, in both extracts, was 40%. Figure 4 shows photographs of the antibacterial test results. To optimize its use as a bactericide, concentrations of 20 to 40% in the extract must be evaluated in order to find the actual effective concentration in bactericidal properties for both synthesis solvents.

(ii) Mercury Removal Capacity of Synthesized AgNPs. Hg(II) removal percentage (%) was calculated to evaluate the efficiency of silver nanoparticles synthesized using either aqueous or ethanolic *Aloe vera* extracts. Figure 5 shows the level of Hg(II) removal for both extracts, using three different initial Hg(II) concentrations and a range of AgNPs concentrations.

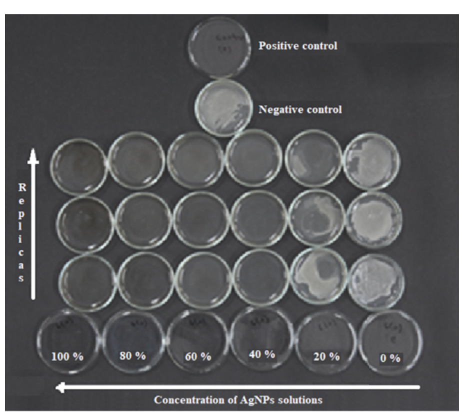


(a)

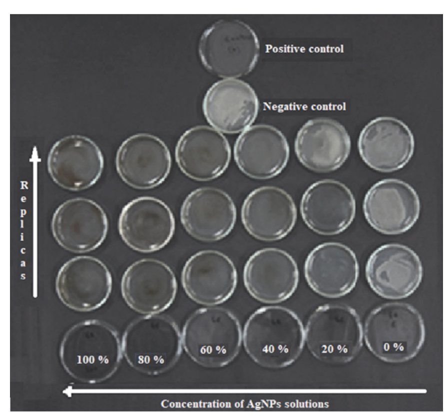


(b)

FIGURE 3: Selected area electron diffraction pattern showing the characteristic crystal planes of elemental silver: (a) aqueous *Aloe vera* extract; (b) ethanolic *Aloe vera* extract.



(a)



(b)

FIGURE 4: Photographs of the antibacterial test results of AgNPs at 100, 80, 60, 40, and 20%, made in triplicate for both (a) aqueous *Aloe vera* extract and (b) ethanolic *Aloe vera* extract.

With a minimal concentration of *Aloe vera* extract (20% V/V), the Hg(II) removal percentage of about 95% was observed for aqueous *Aloe vera* extract and above 96% for ethanolic *Aloe vera* extract, indicating the effectiveness of the two systems. Therefore, it is not necessary to use high concentrations of nanoparticles for effective removal of mercury.

Due to high toxicity effects of mercury, World Health Organization (WHO) has set the limit of mercury in drinking water as 1.0 mg/L (1 ppm) [28] and the United States Environmental Protection Agency (EPA) has set the limit of 2 mg/L (2 ppm) [39]. From the results shown in Figure 5, the maximum amount of mercury remaining unremoved for the

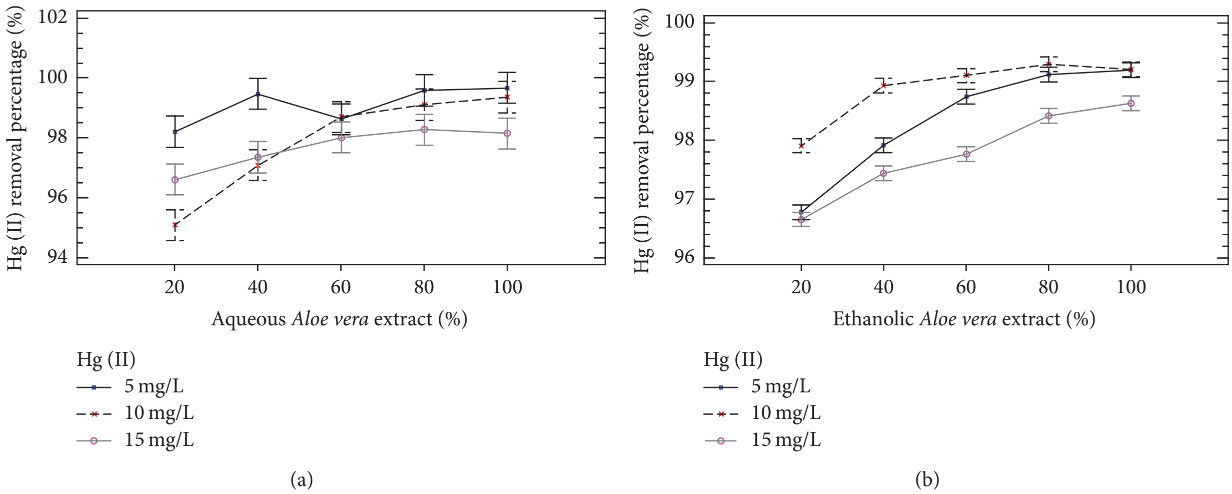


FIGURE 5: Mercury removal percentage at different concentration of Hg(II). (a) Aqueous *Aloe vera* extract and (b) ethanolic *Aloe vera* extract.

20% of concentration of nanoparticles in *Aloe vera* extract is around 0.75 mg/L, which is less than that allowed by the WHO and EPA organizations. Therefore, it is highly feasible to use AgNPs to remediate water contaminated with this metal.

4. Conclusions

Silver nanoparticles (AgNPs) with less than 15 nm size and nearly spherical shape were synthesized using aqueous and ethanolic *Aloe vera* extracts. Crystalline character of AgNPs was evidenced from circular spots in the electron diffraction patterns. The size distribution and lattice constants were analyzed by means of High-Resolution TEM images. These nanoparticles have bactericidal activity against *Kocuria varians*, since only 4% of effective concentration is enough to completely inhibit visible growth. Interestingly, synthesized AgNPs were also used for mercury removal. The Hg(II) removal percentage was above 95% just using 20% V/V concentration of both extracts. This result permitted finding that the maximum amount of mercury that remained unremoved was around 0.75 mg/L, which is below the levels allowed by the WHO and EPA organizations. In this research, the green synthetic method using extracts shown here gives new paths in the development of AgNPs with controlled size and shape. Moreover, due to surface modification of AgNPs by the extracts, new challenges are opened for science (biology, physics, and chemistry) in order to understand the antibacterial mechanism and action, as well as the removal phenomena and its interaction with other metals, among other complex effects.

Finally, the processes on the separation and recovery of AgNPs with Hg are not discussed in this paper. Research on these processes is in progress.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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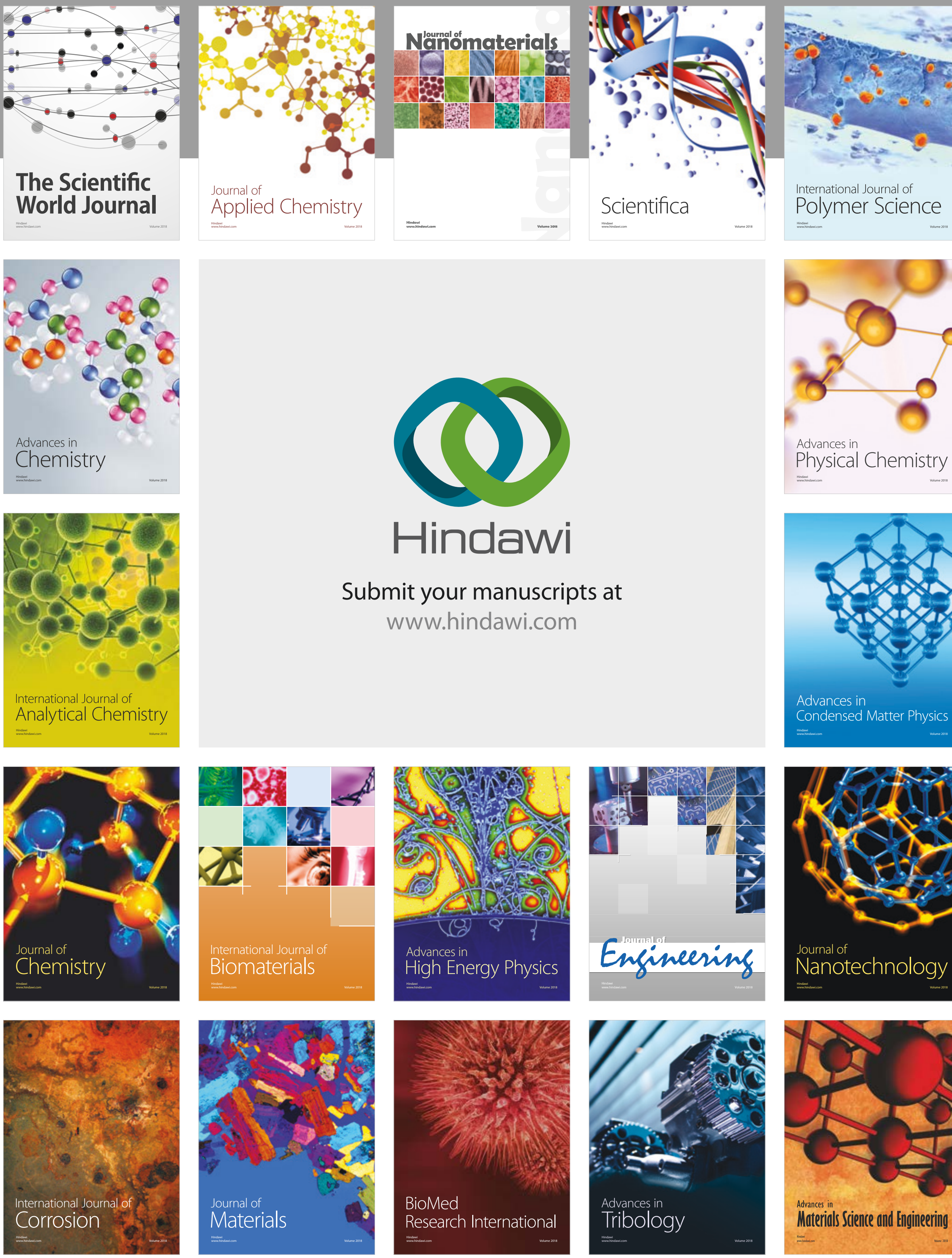

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