Hindawi Publishing Corporation
Journal of Nanomaterials
Volume 2016, Article ID 9204573, 10 pages
http://dx.doi.org/10.1155/2016/9204573

Research Article

Green Synthesis of Silver Nanoparticles and Their Bactericidal and Antimycotic Activities against Oral Microbes

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Received 11 November 2015; Revised 27 March 2016; Accepted 29 March 2016

Academic Editor: Paulo Cesar Morais

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Nanotechnology is a new discipline with huge applications including medicine and pharmacology industries. Although several methods and reducing agents have been employed to synthesize silver nanoparticles, reactive chemicals promote toxicity and nondesired effects on the human and biological systems. The objective of this work was to synthesize silver nanoparticles from *Glycyrrhiza glabra* and *Amphipterygium adstringens* extracts and determine their bactericidal and antimycotic activities against *Enterococcus faecalis* and *Candida albicans* growth, respectively. 1 and 10 mM silver nitrate were mixed with an extract of *Glycyrrhiza glabra* and *Amphipterygium adstringens*. Green silver nanoparticles (AgNPs) were characterized by TEM, Vis-NIR, FTIR, fluorescence, DLS, TGA, and X-ray diffraction (XRD) analysis. Bactericidal and antimycotic activities of AgNPs were determined by Kirby and Bauer method and cell viability MTT assays. AgNPs showed a spherical shape and average size of 9 nm if prepared with *Glycyrrhiza glabra* extract and 3 nm if prepared with *Amphipterygium adstringens* extract. AgNPs inhibited the bacterial and fungal growth as was expected, without a significant cytotoxic effect on human epithelial cells. Altogether, these results strongly suggest that AgNPs could be an interesting option to control oral biofilms.

1. Introduction

Nanotechnology is a relative new discipline with huge applications in many areas including medicine and pharmacology industries [1, 2]. The increasing development of multidrug-resistant strains among pathogen microbes has become one of the most important problems in medicine worldwide [3]. Great advances in nanotechnology have provided a solid foundation for using nanoparticles (NPs) in the fight against pathogen microorganisms, including multidrug-resistant bacteria [2, 4, 5]. Previously the biocidal properties of nanoclusters of several metals have been described, mainly, silver, gold, zinc, titanium, and bismuth [6–10]. Aside from their potential metal nanoparticles present cytotoxicity on human cells, this may occur in all reactive chemicals employed in their synthesis and limiting their use in clinical practice [11–14].

The traditional synthesis of silver nanoparticles (AgNPs) uses chemical reducing agents to modify Ag ions to AgNPs [15]. The most common used chemical reducing agents are ascorbic acid [16], hydrazine [17], and sodium borohydride [18]. The use of these composites involves undesired side effects and the biocompatibility of resulting AgNPs is low for being employed in biological systems. Recently new methods based on green synthesis that use environmental compounds as reducing agents are emerging [19–22]. Plants, bacteria,

fungus, and algae have been used for green synthesis of metal nanoparticles with very good results and water acts as a solvent in the process of green synthesis [23].

AgNPs are synthesized using a green synthesis method. AgNPs are being investigated for application in biomedicine and to be used as bactericide agent or for cancer treatment [24]. Several plants have been used to prepare silver nanoparticles; for example, Alfalfa and *Geranium* (one of the first plants used for these porpoise) produce nanoparticles with sizes of 2–20 nm and 14–46 nm, respectively [25, 26]. Recently, Phanjom et al. (2012) used *Elaeagnus latifolia* leaf extract to synthesize AgNPs with diameters of 30–50 nm [27]; also AgNPs with potential cytotoxic effects against breast cancer cells were prepared using *Ganoderma neojaponicum Imazeki* [28].

The objective of this work was to synthesize silver nanoparticles from *Glycyrrhiza glabra* and *Amphipterygium adstringens* and determine their bactericidal and antimycotic activities against *Enterococcus faecalis* and *Candida albicans* growth, respectively; to our knowledge this is the first report for the green synthesis of silver nanoparticles using these plants. The obtained results show AgNPs of spherical shape and average size lower than 10 nm. As was expected, AgNPs inhibited the bacterial and fungal growth corroborating their biological properties. Altogether, these results strongly suggest that gAgNPs could be an interesting option to control oral biofilms.

2. Material and Methods

2.1. Achievement of Glycyrrhiza glabra and Amphipterygium adstringens Ethanolic Extracts. Bark of *Amphipterygium adstringens* (Cuachalalate) and root of *Glycyrrhiza glabra* were employed to get ethanolic extracts following the method previously described by Viveros-Valdez et al., 2008. 250 g of *A. adstringens* was macerated and put into water in a relation of 50 : 50, $H_2O : C_2H_6O$. In the case of *G. glabra* extract, methanol was employed to dilute the macerated. Both extracts were maintained in continuous agitation for 24 h at room temperature, thus getting 3 extractions, and solvent was eliminated by reduced pressure [29]. A phytochemical analysis of extracts was done to partially identify active ingredients through chemical assays like Shinoda, Liebermann Burchard, coumarins, and so forth [30]. The semicharacterized extracts were stocked at 4°C until being used to synthesize silver nanoparticles.

2.2. Biosynthesis of Silver Nanoparticles, gAgNPs. All reagents were purchased from Sigma-Aldrich Co. and were used without further purification. Deionized water was used to prepare all the aqueous solutions.

2.3. Preparation of the Extract Solution. For the preparation of the extract solutions, a given amount of the dried extract was diluted with dimethyl sulfoxide DMSO to obtain a final concentration of 0.4% (w/v). Once obtained, this solution was stored at 4°C and protected from the sunlight until being used.

2.4. Synthesis of Silver Nanoparticles. Under ambient conditions and magnetic stirring, 100 μL of the extract solution was added to 25 mL of either 1 mM or 10 mM $AgNO_3$ aqueous solution (we obtained 4 different samples, two for the *Glycyrrhiza glabra* extract and two for the *Amphipterygium adstringens* extract; see labels for each sample in Table 2). Immediately after that, the pH value of the reaction mixture was adjusted to 11 using a 3.0 M NaOH solution and then it was stirred for another 15 minutes.

After that time, the pH value of the reaction mixture was again modified to 1.0 using HNO_3 (63%); this modification of pH produces a reversible flocculation of the nanoparticles, allowing us to easily separate them from the subproducts of the reaction by centrifugation at 6000 rpm. The supernatant was discarded and the pellet was redispersed using deionized water and, if necessary, the pH was adjusted to 7.0.

To obtain dried powders of the nanoparticles, after the centrifugation, the separated pellet was dried overnight in a conventional oven at 60°C.

2.5. Characterization of the Silver Nanoparticles. The morphology and size of the silver nanoparticles were analyzed using transmission electron microscopy (TEM) with a JEOL JEM1230 microscope operating at an acceleration voltage of 100 kV. TEM images of the prepared nanoparticles were used for the size distribution measurements; the size of 300 particles was measured to obtain the average size. Vis-NIR absorption spectra were obtained using a CHEMUSB4-VIS-NIR (Ocean Optics) spectrophotometer. Hydrodynamic diameter and zeta potential were measured using dynamic light scattering (DLS) with a Zetasizer Nano ZS (Malvern Instruments) operating with a He-Ne laser at a wavelength of 633 nm and a detection angle of 90° at 25°C. Fluorescence emission (FLUO) was observed using a USB4000 spectrophotometer (Ocean Optics); samples were excited with a solid state continuous laser (532 nm, 100 mW). All of the above characterization techniques were performed using the aqueous dispersion of the nanoparticles.

Using the dried powders, X-ray diffraction (XRD) patterns were obtained on a GBC-Difftech MMA model, with a Cu Kα irradiation at $\lambda = 1.54$ Å; thermogravimetric analysis (TGA) was performed in a DSC500 calorimeter (Waters), using a constant heating rate of 2°C/min from room temperature to 1000°C; infrared spectra (IR) were obtained with a FTIR spectrophotometer (IRAffinity, Shimadzu) from 400 to 4000 cm^{-1}.

2.6. Antimicrobial Activity of AgNPs by Kirby and Bauer Method and Cell Viability MTT Assay. The bactericidal and antifungal activities of AgNPs on *Enterococcus faecalis* and *Candida albicans* growth (ATCC numbers: 11420 and 90029) were determined using Kirby and Bauer disk diffusion method [31] and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Biotium, Hayward, CA) [32] following the instructions of the manufacturer. *E. faecalis* and *C. albicans* were grown in trypticase soy broth (TSB, BD DIFCO, Sparks, MD, USA) at 37°C, overnight in aerobic conditions. 100 μL of microbial culture was spread

on TSB agar plate using a sterile cotton swab. AgNPs were embedded into the agar plate to interfere with bacterial growth. The Petri plate was incubated at 37°C overnight and the inhibition of bacterial growth was directly proportional to diameter of halo measured with a Vernier. Experiments were repeated three times to assess the veracity of results.

The cell viability was evaluated by the amount of viable cells stained by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Biotium, Hayward, CA) which was released with dimethylsulfoxide (DMSO, Sigma-Aldrich Inc., St. Louis, MO, USA). *E. faecalis* and *C. albicans* were grown in trypticase soy broth (TSB, BD DIFCO, Sparks, MD, USA) at 37°C, overnight in aerobic conditions. The microbial cells were counted using a Neubauer chamber and 1×10^4 cells were inoculated in 100 μL of trypticase soy broth medium in a 96-well polystyrene plate. Three wells with only trypticase soy broth medium were used as controls for microbial growth and AgNPs at final concentration of 0.1 and 1 mM were added. 1.2 mM of chlorhexidine (Ultradent Products, South Jordan, UT) and 0.2 mM of colloidal silver (Microdyn, Tavistock, B) were employed as positive inhibitors of bacterial growth, while 0.002 mM of amphotericin B (Pisa, Mexico) and 0.2 mM of colloidal silver were used as positive inhibitors of fungal growth. AgNPs at final concentration of 1 and 10 mM were used to assess their antimicrobial activity. The 96-well plate was incubated at 37°C overnight. Next, 10 μL of MTT was added to each well to get a final concentration of 0.1 mg/mL; the plate was protected against light and incubated at 37°C for 2 h. Next, 200 μL of dimethyl sulfoxide was added to dissolve the reduced MTT species. The amount of cells alive was determined using a microplate absorbance reader (Biorad, Philadelphia, PA) at 570 nm. The experiments were repeated three times and the measured optical density was analyzed by descriptive statistics.

2.7. Cytotoxicity of Silver Nanoparticles Biosynthesized with G. glabra and A. adstringens Ethanolic Extracts. In order to get insight into possible nondesired effects of AgNPs, in this research we used human epithelial cells (HeLa) to analyze the cytotoxic effects of AgNPs. A confluent monolayer of 1×10^5 HeLa cells (ATTCC Nro. CCL-2) was grown in Minimal Essential Medium (MEM, Invitrogen, Carlsbad, CA) supplemented with 10% of Fetal Bovine Serum (FBS, Invitrogen, Carlsbad, CA) at 37°C with 5% of CO_2 in 96-well microtiter plates (Thermo Fisher Scientific Inc.). It was exposed to 1–0.015 mM of AgNPs for 24 h, and the possible cytotoxic effect was detected by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Biotium, Hayward, CA) [32]. As negative control, cells without nanoparticles were employed. 10 μL of MTT was added to each well; the plate was protected against light and incubated at 37°C for 2 h. Next, the medium was removed carefully and then 100 μL of dimethyl sulfoxide was added to dissolve the reduced MTT species. The amount of cells alive was determined using a microplate absorbance reader (Biorad, Philadelphia, PA) at 570 nm. The experiments were repeated three times and the measured optical density was analyzed by descriptive statistics.

Table 1: Phytochemical analysis of ethanolic extracts of *Glycyrrhiza glabra* root and *Amphipterygium adstringens* bark.

Phytochemical component	*Glycyrrhiza glabra*	*Amphipterygium adstringens*
Alkaloids	ND	ND
Flavonoids	+	ND
Carbohydrates	+	+
Saponins	ND	ND
Tannins	+	+
Double bonds	+	+
Carbonyl groups	+	+
Sterols and triterpenes	+	+
Coumarins	+	+
Sesquiterpene lactones	ND	ND
Quinones	ND	ND

+: identified; ND: not detected.

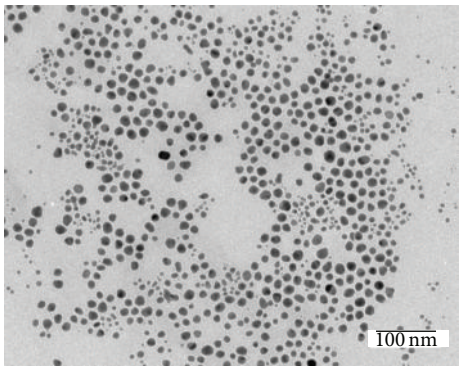
3. Results

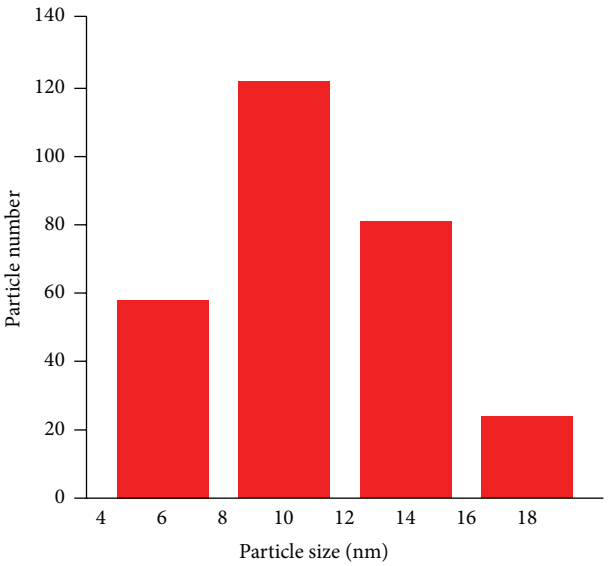
3.1. Glycyrrhiza glabra and Amphipterygium adstringens Ethanolic Extracts. The phytochemical screening of extracts showed the presence of tannins, sterols, triterpenes, and coumarins (Table 1). Flavonoids were detected in *G. glabra* extract but they were undetectable in *A. adstringens* extract. This result is very interesting due to early reports indicating a clear correlation between strong antioxidant activity and phenolic and flavonoids contents [33]. In theory, the major content in flavonoids in *G. glabra* extract could reduce silver nitrate more successfully than *A. adstringens* extracts.

3.2. Synthesis of Nanoparticles. The addition of the extract to the $AgNO_3$ solution produces a change in color from colorless to pale yellow; when we added the NaOH solution the reaction mixture immediately changes from pale yellow to light brown (for the 1 mM solutions) and to black (for the 10 mM solutions); this change in color is an indicator of the immediate formation of the nanoparticles; the addition of NaOH in a green synthesis has already been reported by Singh et al. (2009); they also used NaOH as an accelerator of the reaction [34].

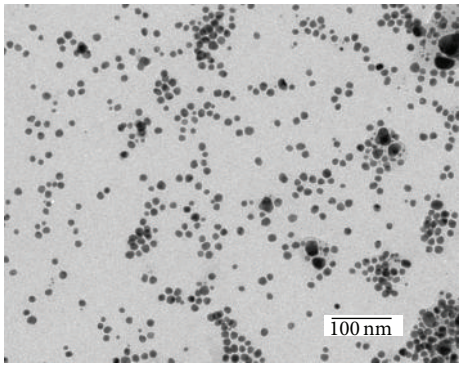
During the next 15 minutes of stirring an Ostwald ripening process occurs and allows the population of nanoparticles to be more homogeneous in size. No important changes in the properties of the silver nanoparticles were observed when the concentration of the silver nitrate solution changed.

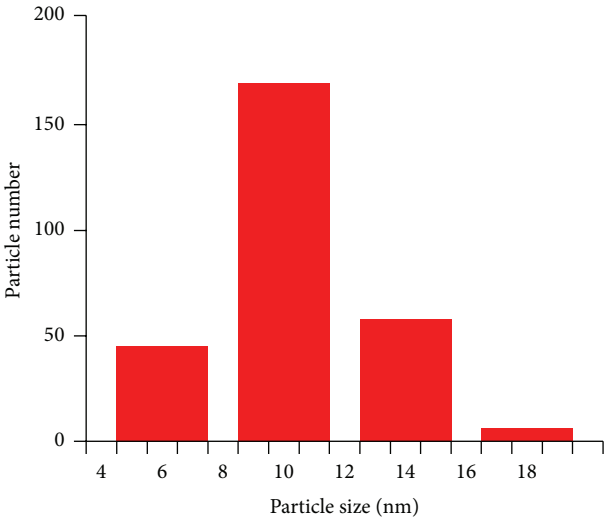
3.3. Characterization of Silver Nanoparticles. Figure 1 presents TEM images and histograms of the four samples obtained in this work; all of them have a narrow particle size distribution and spherical morphology. As we can see, samples prepared using the Gg extract are bigger than samples prepared using the Aa extract (see Table 2). Table 2 also presents the results of the DLS analysis; it can be observed that the hydrodynamic size of all samples is bigger probably due to the presence of biomolecules in their surface (the presence of these biomolecules was further confirmed by FTIR and



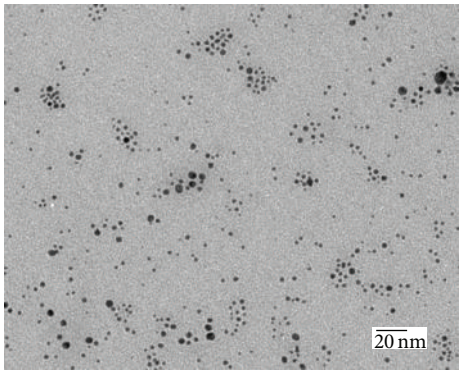


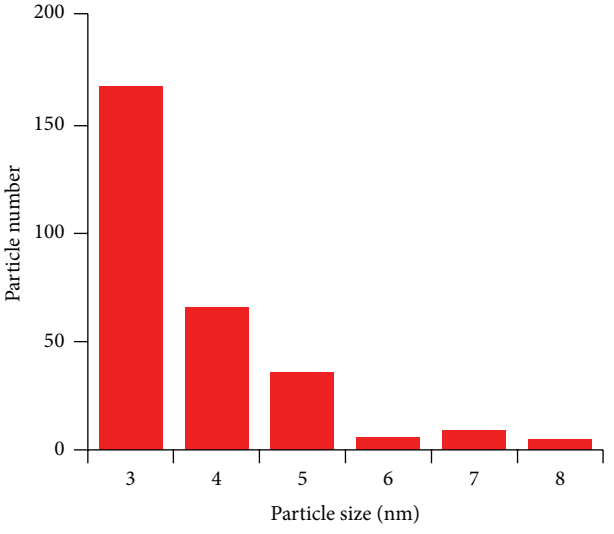
(a)





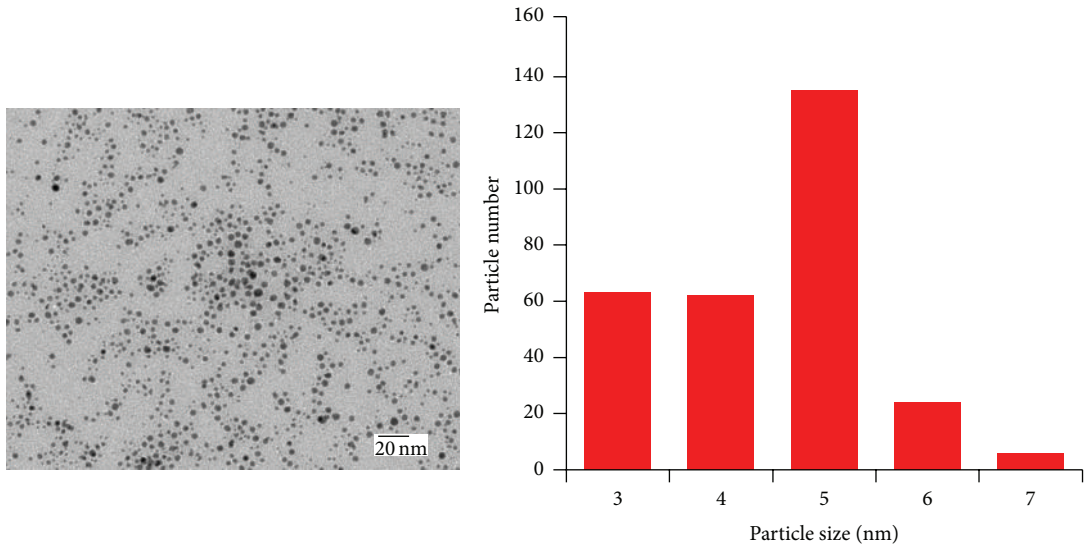
(b)





(c)

Figure 1: Continued.



(d)

Figure 1: TEM images and histograms for size distribution of silver nanoparticles biosynthesized from *Glycyrrhiza glabra* and *Amphipterygium adstringens*. (a) Sample AgG1, (b) sample AgG10, (c) sample AgA1, and (d) sample AgA10.

Table 2: Size and zeta potential of the four samples prepared in this work.

Sample*	Size TEM M ± SD (nm)	Hydrodynamic diameter M ± SD (nm)	Zeta potential (mV)
AgG1	9.7 ± 3.8	25.39 ± 3.89	−42.5 ± 11.2
AgG10	9.5 ± 2.9	7.892 ± 0.82	−31.9 ± 9.12
AgA1	3.1 ± 1.2	133.7 ± 24.16	−50.9 ± 10.3
AgA10	3.9 ± 1.1	42.69 ± 7.24	−42.3 ± 7.52

*G means samples prepared with *Glycyrrhiza glabra* and A means samples prepared with *Amphipterygium adstringens*. 1 and 10 are for samples prepared with $AgNO_3$ at 1 and 10 mM, respectively.

TGA analyses); potential zeta values range from −31.9 mV to −50.9 mV.

The Vis-NIR spectra are presented in Figure 2; differences in size are not reflected in the position of the surface plasmon resonance (SPR); all samples have the characteristic SPR of silver nanoparticles at 420 nm; these spectra are narrow reflecting the narrow particle size distribution of the samples; it has been reported that the nature of the extract modifies the position of the plasmon and similar results were reported for nanoparticles synthesized with *Chrysopogon zizanioides*, *Prosopis juliflora*, *Moringa oleifera*, and *Indigofera aspalathoides* [35].

Figure 3 shows the diffraction patterns of the samples obtained at 10 mM $AgNO_3$ solution for both extracts; samples can be indexed as pure metallic silver (JCPDS 04-0783) without the presence of any other phases; broader peaks can be seen for sample AgA10 due to the lower particle size and probably due to a lower crystallinity.

FTIR spectra are shown in Figure 4; we found peaks for both samples (AgA and AgG) at 1015 cm^{-1} (C-N stretch, aliphatic amines), 1385 cm^{-1} [C-C stretch (in-ring), aromatics], 1595 cm^{-1} (N-H bend, primary amines), 1737 cm^{-1} (C=O stretch; esters, saturated aliphatic), 1990 cm^{-1} (-C≡C- stretch, alkynes), 2849 cm^{-1} (H-C=O: C-H stretch, aldehydes), 2920 cm^{-1} (C-H stretch, alkanes), 3302 cm^{-1} only for G extract (O-H stretch, H-bonded; alcohols, phenols), and 3758 cm^{-1} (O-H stretch, primary alcohol) only for G extract; similar results were reported by [36, 37].

As a remarkable feature of the nanoparticles prepared with the extracts of both *A. adstringens* and *G. glabra* we found that when irradiated with a 532 nm wavelength laser, they present an emission band with a maximum at 580 nm (Figure 5). This emission band is most probably coming from the extract molecules in the surface of the silver nanoparticles because it has been reported that only ultrasmall metal nanoclusters could present this property intrinsically [38].

3.4. Antimicrobial Activity of AgNPs by Kirby and Bauer Method and Cell Viability MTT Assay. In order to determine if the biosynthesized silver nanoparticles have bactericidal and antimycotic activities Kirby and Bauer disk diffusion assays were performed. The results showed that inhibition of *Enterococcus faecalis* and *Candida albicans* growth with AgNPs developed with both extracts (samples AgA10 and AgG10) as can be seen in Figure 6 with average inhibition zone of 10 mm. Samples AgA1 and AgG1 did not show a relevant inhibition of microbial growth; this may be due to incomplete reduction of silver nitrate or due to the extract molecules surrounding the surface of the nanoparticles causing agglomeration and passivation (see Figure S1 in Supplementary Material available online at http://dx.doi.org/10.1155/2016/9204573). These results were expected based on the size and morphology of AgNPs, since spherical morphology, concentration, and low size are tightly related to their biological action. With the intention of corroborating the antimicrobial activity of AgNPs, cell viability MTT assays were carried out. Again silver nanoparticles

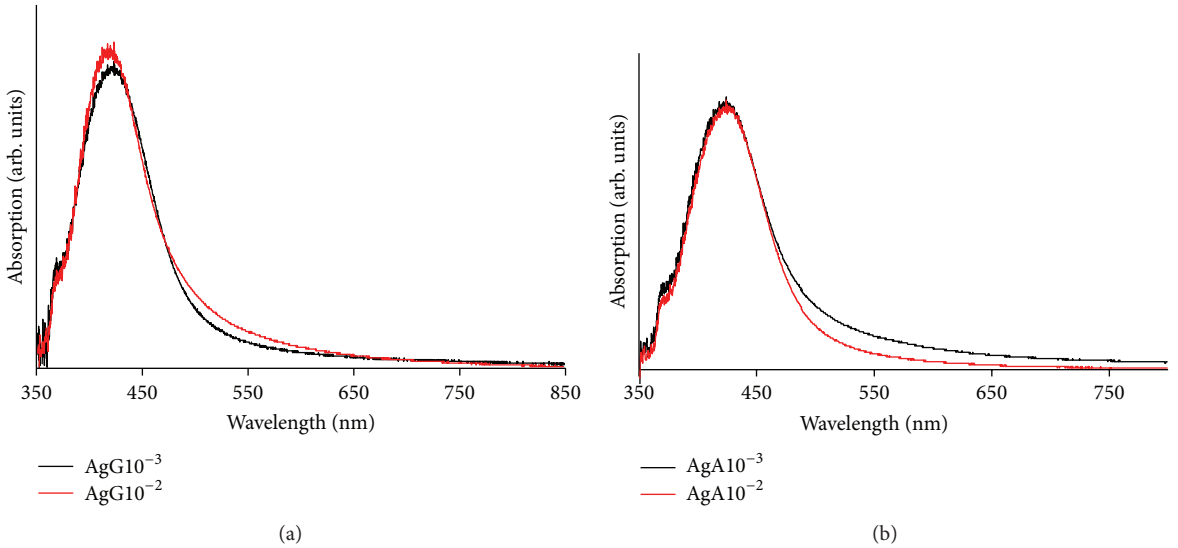


Figure 2: Vis-NIR spectra of the silver nanoparticles. (a) Samples prepared with Gg extract and (b) samples prepared with Aa extract. The characteristic surface plasmon resonance is located at 420 nm for each sample.

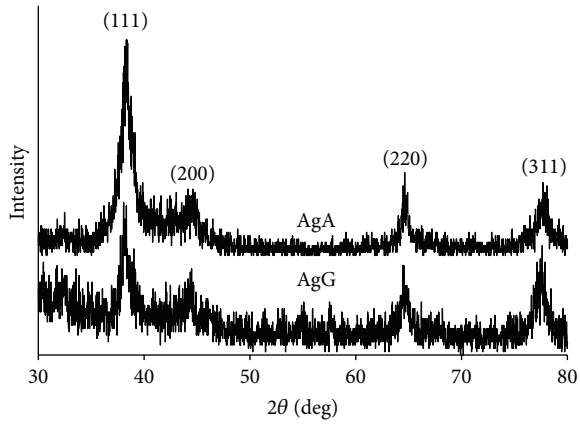


Figure 3: X-ray diffraction patterns of the samples obtained at the higher concentrations of $AgNO_3$. Samples were identified as pure silver.

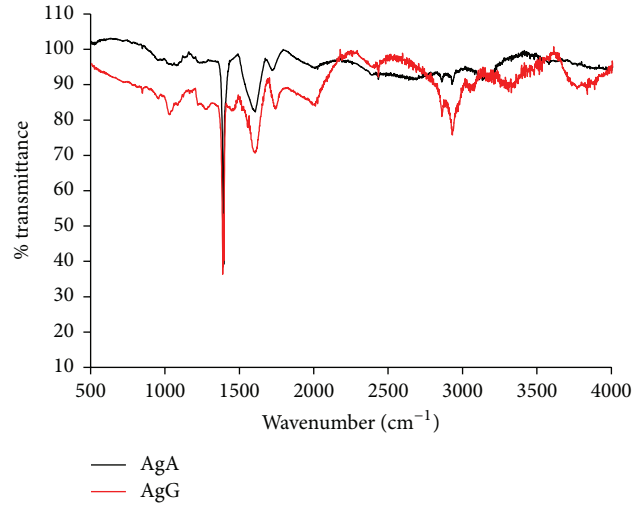


Figure 4: FTIR analysis of the functional groups adsorbed on the surface of the silver nanoparticles.

exhibited bactericidal and fungicidal activities, decreasing the microbial growth until 90% (Figures 6 and 7). To compare the bactericidal activity of silver nanoparticles 1.2 mM of chlorhexidine and 0.2 mM of colloidal silver were employed, while for antimycotic activity 2.5 μg/mL of B amphotericin and 0.2 mM of colloidal silver were used. 1 mM of silver nanoparticles inhibited 90% of bacterial growth, while 76% and 41% were blocked by chlorhexidine and colloidal silver, respectively. 1 mM of silver nanoparticles inhibited 78% of fungal growth while amphotericin B and colloidal silver interfere with 81% and 41% of fungal growth. In summary, silver nanoparticles synthesized by green method compete in efficacy with commercial products.

3.5. Cytotoxicity of AgNPs on Human Epithelial Cells. The cytotoxic effect of biosynthesized silver nanoparticles was evaluated in human epithelial cells by cell viability MTT assay. The obtained results indicate that silver nanoparticles developed with *A. adstringens* extract were more toxic to human cells instead of nanoparticles synthetized with *G. glabra* extract (Figure 8). The cytotoxic potential of silver nanoparticles on human cells [11, 15] is well described; however, silver nanoparticles synthesized with *G. glabra* extract showed less side effects and they exhibited a good antimicrobial activity. In summary, side effects and antimicrobial activity must be evaluated before a possible clinical use of this kind of nanoparticles.

4. Discussion

Nanotechnology is a relative new discipline with high potential in pharmaceutical industry and biomedical sciences

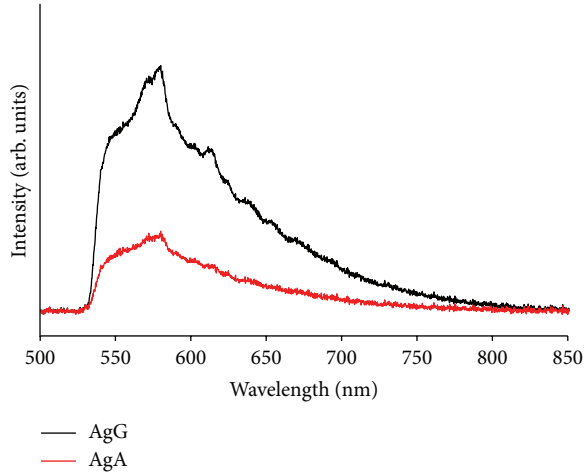


Figure 5: Emission spectra of AgNPs prepared in this work. A maximum at 580 nm can be observed. The excitation was done at 532 nm, 100 mW power.

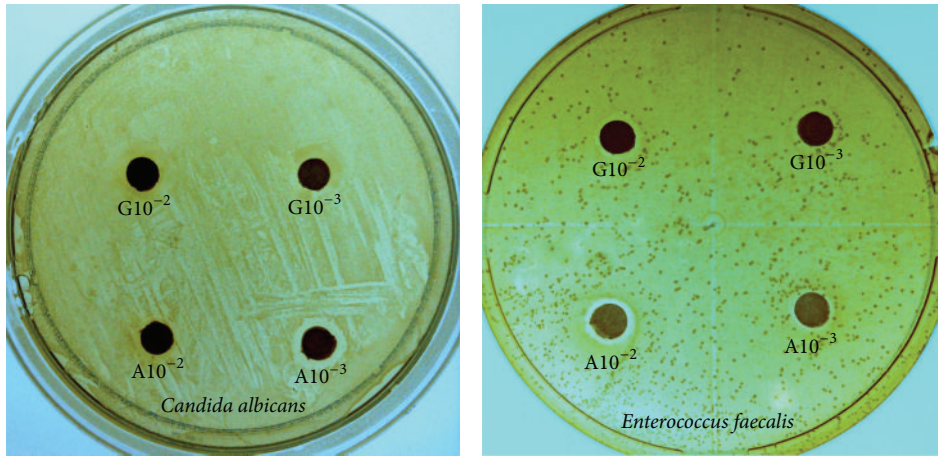


Figure 6: Antimicrobial activity of silver nanoparticles biosynthesized against *Enterococcus faecalis* and *Candida albicans* growth. The antimycotic activity of AgNPs was tested against *C. albicans* and bactericidal activity against *E. faecalis* employing Kirby and Bauer diffusion disk method. The average diameter halo of microbial inhibition is proportional to the activity of AgNPs. G means *Glycyrrhiza glabra* and A *Amphipterygium adstringens,* respectively, diluted 10^{-2} or 10^{-3}.

[39–43]. Nanotechnology applied to medicine tackles fundamental problems like cancer treatment, multiresistance among pathogen microorganisms, vaccines, biotechnology devices, molecular machine systems, and so forth. Nanoparticle-based therapy would be the evolutionated medical treatment with "smart" therapeutics that is simultaneously more potent with less toxic drugs [5]. Metallic nanoparticles are frequently developed by chemical and physical methods [44]. However, in order to look for ecofriendly and green synthesis of metallic NPs diverse biological entities like microorganisms, plant extracts, and enzymes are being employed [44].

In this work we describe a green method to synthesize silver nanoparticles employing *Glycyrrhiza glabra* and *Amphipterygium adstringens* ethanolic extracts; to our knowledge this is the first report for the green synthesis of silver nanoparticles using extracts of these plants. The name and structure of the constituents in the ethanolic extracts of *Glycyrrhiza glabra* and *Amphipterygium adstringens* have been reported elsewhere; for *Glycyrrhiza glabra*, Alwan et al. (2015) [45] reported glycyrrhizic acid as the main biologically active component and triterpene saponins, flavonoids, isoflavonoids, and chalcones as secondary components; molecular structures of these compounds can be found in the review of Asl and Hosseinzadeh (2008) [46–48]. For *Amphipterygium adstringens*, Navarrete et al. (2005) [49] mention triterpenoids and long-chain phenols as the major components of the methanolic extracts; molecular structures can be found in Khadem and Marles (2010) [47, 50, 51].

In our work, in addition to the molecules already reported we found the presence of tannins (see Table 1); tannins are polyphenolic molecules present in most natural extracts.

Although it is possible that all molecules in the extracts play a role in the nucleation of silver nanoparticles we believe that tannins play the major role. In our procedure (for both extracts) the nucleation begins with the addition of

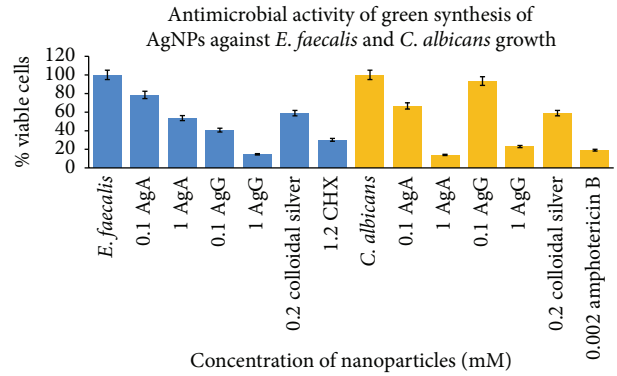


Figure 7: Antimicrobial activity of AgNPs against *Enterococcus faecalis* and *Candida albicans* growth. The y-axis shows the percent of viable cells. As growing control of bacteria or fungus culture media were added. AgNPs synthesized with Aa or Gb extracts were used at final concentrations of 0.1 and 1 mM. 1.2 mM of chlorhexidine and 0.2 mM of colloidal silver were employed as positive inhibitors of bacterial growth, while 0.002 mM of amphotericin B and 0.2 mM of colloidal silver were used as positive inhibitors of fungal growth. The bactericidal and antimycotic activities of AgNPs were measured after 18 h after exposition. All experiments were done by triplicate to assess the veracity of results.

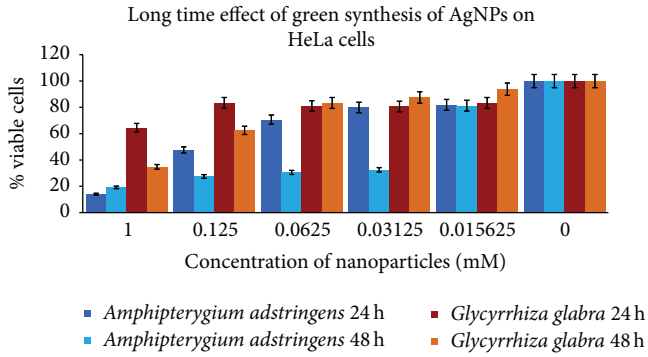


Figure 8: Cytotoxicity of silver nanoparticles determined by cell viability MTT assays. The possible cytotoxic effect of AgNPs synthesized with different concentrations of Aa or Gg extracts was evaluated in HeLa cells. As positive control HeLa cells growing in culture media were used. All experiments were done in triplicate to assess the veracity of results.

NaOH; this change in the pH hydrolyzes tannins into glucose and gallic acid; once gallic acid is formed the reduction of silver ions is achieved by the oxidation reaction of phenol groups. Following the results of the infrared analysis we propose that tannins, flavonoids, and carbohydrates take part in the stabilization process of silver nanoparticles. Silver nanoparticles obtained were spherical in shape with an average size under 10 nm; here, flavonoids and tannins are the molecules responsible for the reduction and stabilization of the nanoparticles through their -OH groups. These results are similar to AgNPs obtained through green synthesis employing *Artemisia absinthium* aqueous extract and bark extracts of *Ficus benghalensis* and *Azadirachta indica* as reductor agents [48, 50]. Their efficacy in inhibiting the *Enterococcus faecalis* and *Candida albicans* growth was similar to that obtained with chemical methods [18], competing against commercial products even better than colloidal silver. AgNPs synthesized with *G. glabra* show more antimicrobial activity than nanoparticles developed with *A. adstringens*. Previous reports indicate a clear correlation between strong antioxidant activity and phenolic and flavonoids contents [33] and the phytochemical analysis showed the presence of flavonoids only in *G. glabra* ethanolic extracts. These flavonoids and the major quantity of the *G. glabra* extract in the surface of the nanoparticles (see Figure S2) compared with those prepared with the *A. adstringens* extract could be responsible for the differences in the antibacterial activity.

It would be interesting to try the synthesis of AgNPs with purified flavonoids and compare the biocidal activity of the obtained nanoparticles.

When cytotoxicity of AgNPs was explored on human epithelial cells, the results showed that the addition of AgNPs developed with *A. adstringens* extract was more toxic to human cells instead of nanoparticles synthetized with *G. glabra* extract (Figure 8). Several reports described toxic effect of silver nanoparticles on human cells, limiting their use in clinical treatments [15]. However, green synthesis did not exclude cytotoxicity in silver nanoparticles like Safaepour report in 2009 using *Pelargonium graveolens* and *Azadirachta indica* as reductor agents [51]. More studies are needed to understand the complete effects of employing biological systems to biosynthesize metallic nanoparticles.

5. Conclusions

In summary, silver nanoparticles biosynthesized with *Glycyrrhiza glabra* root and *Amphipterygium adstringens* bark extracts inhibited the bacterial and fungal growth suggesting that green synthesis could be an interesting option to develop silver nanoparticles with biocidal properties.

Competing Interests

The authors of this paper declare that there are no potential competing interests.

Acknowledgments

The authors wish to thank CONACyT for financing Projects 169020, 178261, and 183825. Financial support for payment of the processing charges was made by Programa para el Desarrollo Profesional Docente para el Tipo Superior (PRODEP 2014 and 2015). N. Niño-Martínez wants to thank Fondo de Apoyo a la Investigación (FAI, UASLP 2015, Grant C15-FAI-04-77.77) and PRODEP 2015 (Grant DSA/103.5/15/6988).

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