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Original Article

Rapid green synthesis of silver nanoparticles and evaluation of their properties for oral disease therapy

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Abstract

A rapid, cost-efficient, and eco-friendly method for synthesis of silver nanoparticles (AgNPs) was developed using an herb extract (*Glycyrrhiza glabra* root) as a reducing agent and a kitchen microwave as a reaction accelerator. A solution of dark brown appeared and was examined by UV-visible spectroscopy. Scanning and transmission electron microscopes as well as an atomic force microscope were used to confirm their physical nature. The AgNPs revealed feasibility for treatment of oral diseases. Their antimicrobial activities against *Streptococcus mutans* were indicated with minimal inhibitory and minimal bactericidal concentrations of 6.25 and 25 µg/ml, respectively. They showed toxicity to cancer cell lines (HN-30) but had no effect on human gingival fibroblasts. Anti-oxidative activity was also observed which suggested potential in medical applications.

Keywords: silver nanoparticles, *Glycyrrhiza glabra*, *Streptococcus mutans*, antimicrobial, cytotoxicity, anti-oxidative

1. Introduction

Silver is a noble metal and has long been known to possess the property of inhibiting or killing microorganisms (Bindhu & Umadevi, 2015; Sharma *et al*., 2009; Sondi & Salopek-Sondi, 2004). Its antimicrobial activity in the form of nanoparticles had been extensively studied and applied, for example, by coating on medical devices, textiles or mixing in cosmetics (Cheng *et al*., 2006; Duránj *et al*., 2007; Ge *et al*., 2009; Jayalakshmi *et al*., 2006; Jie *et al*., 2010; Montazer *et al*., 2012; Porter & Youtie, 2009). Silver nanoparticles (Ag NPs) can be synthesized by a non-toxic, eco-friendly, and publicly accepted process known as biosynthesis. This process uses biomaterials such as proteins (Morales-Sanchez *et al*., 2011), peptides (Nam *et al*., 2008), amino acids (Perni *et al*., 2014), polysaccharides (Anisha *et al*., 2013; Ortega *et al*., 2013), bacteria species (Garmasheva *et al*., 2016), bacterial products (Shahverdi, *et al*., 2007), fungi (Balaji *et al*., 2009), algae (Azizi *et al*., 2014; Sinha *et al*., 2015), and plants (Mukunthan *et al*., 2011; Patil *et al*., 2012; Tripathi *et al*., 2009). These biomaterial products can reduce Ag^{+} (ions) from AgNO3 to Ag particles (Morales-Sanchez *et al*., 2011; Nam *et al*., 2008). Compared to materials used in a physical or chemical process, the reducing or capping agent in the particle production step is mainly derived from plants or generated by microorganisms which produces fewer polluting substances (Ahmed *et al*, 2016; Banerjee *et al*., 2014; Ge *et al*., 2014). The medicinal plant extracts are cheap, easily available, and safe for humans and the environment (Banerjee *et al*., 2014). The root of *Glycyrrhiza glabra* (*G. glabra*) or lico rice had been found to have antimicrobial (Ahn *et al*., 2015; Ghannad *et al*., 2014; Krausse *et al*., 2004; Masoomeh & Kiarash, 2007), anti-inflammatory (Nirmala & Selvaraj, 2011; Racková *et al*., 2007), anticancer (Sheela, *et al*., 2006; Shibata, 1994), and anti-oxidative activities (Monica, 2014; Morteza-Semnani

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et al., 2003). With its extract, AgNPs can be produced and their antimicrobial activity against *Helicobacter pylori* (*H. pylori*), the causative agent of gastric ulcer, was recently reported (Dinesh *et al*., 2012; Sreelakahmy *et al*., 2016). The production of AgNPs can be sped up using microwaves as the heating source or as a reaction accelerator (Chen *et al*, 2008; Hu *et al*., 2008). In this study, *G. glabra* root extract was used to synthesize AgNPs by a microwave-assisted process. The size and form of the AgNPs was imaged and measured, and their properties for oral disease therapy were evaluated.

2. Materials and Methods

2.1 Test microorganism

Streptococcus mutans A32-2 (*S. mutans*) was kindly provided by Prof. R. L. Gregory, School of Dentistry, Indiana University, USA. Bacterial cultures were grown overnight on nutrient agar (NA) or in nutrient broth (NB) before testing.

2.2 Preparation of *G. glabra* root extract

G. glabra root extract was purchased from the Thai-Chinese Flavor Company (Thailand). The crude extract was achieved according to the following procedure. A 250 g portion of *G. glabra* root powder was dissolved in 100 ml of 50% ethanol. The suspension was then extracted using an evaporator with continuous agitation at room temperature for 24 h. The extract was kept at 4 °C until use.

2.3 Synthesis of AgNPs using G. glabra extract

AgNPs were synthesized using silver nitrate ($AgNO_3$) from Sigma Aldrich® as a substrate and *G. glabra* extract as the reducing or capping agent. To find the optimal condition to generate AgNPs, various concentration ratios of $AgNO_3$ and *G. glabra* extract were tested. A solution of $AgNO_3$ was mixed with the extract to make final concentrations between $AgNO_3$ (mM) and *G. glabra* extract (mg/ml) of 1:0.1, 1:0.5, 1:1, 1:2, 1:3, and 1:4. All solutions with a volume of 20 ml were then heated in a microwave (800 W [medium power]) for 2 min to accelerate the reactions in which Ag^+ was reduced to Ag, hence forming AgNPs. Synthesized AgNPs in the extracts were subsequently characterized and measured for their biological properties.

2.4 Analysis of synthesized AgNPs

The UV-visible spectra of the $AgNO_3$/*G. glabra* root extract mixtures both before and after heating for 2 min were recorded as a function of wavelength using a UV-vis spectrophotometer (Helios Gamma, Thermo Corporation, England) operated at a resolution of 0.5 nm. Absorbance patterns of synthesized AgNPs from various concentration ratios were also compared. The shapes and sizes of the AgNPs were determined by scanning electron microscopy (SEM) (LEO 1455VP, USA) and transmission electron microscopy (TEM) (PHILIPS Tecnai12, Netherlands). Their morphologies in 3-dimensional fields were confirmed by atomic force microscopy (AFM) (AGILENT-N9410A series 5500).

2.5 Analysis of antimicrobial activities

Antimicrobial activities of synthesized AgNPs were investigated by means of agar well diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays.

2.5.1 Agar well diffusion assay

The test microorganisms were swabbed uniformly on the NA plates. Five wells of 6-mm diameter were made using a sterile well borer and 20 µl of AgNPs solutions with various concentrations were pipetted into the corresponding wells. Chlorhexidine (0.2% w/w) and $AgNO_3$ solutions (1 mM) were also used as controls. The culture plates were then incubated at 37 °C for 24 h. After incubation, the diameters of the inhibition zones for each well were measured.

2.5.2 Determinations of MIC and MBC

Flasks containing 50 ml of sterile NB were supplemented with various concentrations of AgNPs. Each was 2-fold serially diluted from a 1:2 concentration ratio of Ag NO_3/*G. glabra* root extract, thus giving actual concentrations that ranged between 3.25 and 1000 µg/ml. All flasks were inoculated with 0.1 ml of a test microorganism (OD_{600}=0.8) and then incubated in shaking incubator (150 rpm) at 37 °C for 24 h. AgNPs-free NB was used as a control sample. Microbial growth was measured using a UV-vis spectrophotometer (OD_{600}). The lowest concentration of AgNPs that gave an absorbance value similar to the control sample was considered as the MIC. For the MBC assay, a loopful of each microbial culture grown in MIC flasks was inoculated onto the AgNPs-free NA plates and incubated under the same conditions. The lowest concentration of AgNPs that prevented microbial growth was designated as MBC.

2.6 Analysis of cytotoxicity

2.6.1 Cytotoxicity to human head and neck squamous cell carcinoma

1) Cell culture

Human head and neck squamous cell carcinoma (HNSCC) cell line HN-30 was used based on their known patterns of genetic aberration and clinical data of tumors. Cell line HN-30 was kindly provided by Prof. J. S. Gutkind (NIDCR, NIH, USA). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fatal bovine serum (FBS), L-glutamine (2 mM), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) (Gibco®, USA), and then incubated at 37 °C in a humidified atmosphere of 95% air and 5% CO_2.

2) MTT assay

The cytotoxic effects of synthesized AgNPs on HNSCC cells were determined by 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. HN-30

cells were seeded into 24-well culture plates. Each well (~$5x10^4$ cells/well) contained serum-free DMEM supplemented with L-glutamine (2 mM), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml). Seeded cells were treated for 24 h with various concentrations of AgNPs (10-fold serially diluted from 1:2 concentration ratio of $AgNO_3$/*G. glabra* root extract, giving the actual concentration of 1, 10, and 100 µg/ml). Before termination, the cultured medium was aspirated and replaced with 0.5 mg/ml of MTT solution and then stored at 37 °C for 30 min in a CO_2 incubator. The reaction was measured spectrophotometrically (OD_{540}) using a microplate reader (Bio-Rad®) and calculated as viable cell numbers. The growth inhibition ratio was calculated according to the following equation:

$$\text{Inhibition ratio (\%)} = (\text{Control group} - \text{Treated group})/\text{Control group} \times 100 \quad (1)$$

2.6.2 Cytotoxicity to human gingival fibroblasts

Human gingival fibroblasts (HGF) were used to determine the cytotoxicity of the synthesized AgNPs using the CellTiter 96 Aqueous One Solution Cell Proliferation Kit (MTT assay; Promega®). The HGF cells were seeded into 24-well plates. Each well (~$5x10^4$ cells/well) contained DMEM supplemented with 10% FBS, penicillin (100 IU/ml), streptomycin (100 µg/ml), and amphotericin B (5 µg/ml) (Hyclone®, USA). After 24 h of incubation, serum medium was poured out. Cells in each well were then treated for another 24 h with various concentrations of AgNPs as in the previous experiment. To detect the effect of AgNPs on the HGF, the mixtures in each well was replaced with 100 ml of serum-free medium and 20 ml of MTT solution. The reaction was left at 37 °C for 1-4 h before spectrophotometric (OD_{540}) measurements were made using a microplate reader (Perkin Elmer®). The results were then calculated and presented as viable cell numbers. All measurements were performed in triplicate and repeated in 3 different occasions.

2.7 Analysis of anti-oxidant activity

Free radical scavenging activity of the AgNPs was evaluated *in vitro* using the modified DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay (Goodarzi *et al.*, 2014). Various concentrations of AgNPs (10, 50, 100, 250, 500, and 1000 µg/ml) were calculated and prepared from the mixture ratio of 1:2. Each was mixed with DPPH (2 ml, 400 mM in 80% v/v ethanol) in an eppendorf tube. The mixture was centrifuged at 10,000 rpm for 2 min. Following centrifugation, the supernatant was measured spectrophotometrically (OD_{517}). Ascorbic acid (10 µg/ml) was used as the positive control. All measurements were carried out after a 30-min mixing of insoluble nanoparticles with the DPPH reagent. The low absorbance of the reaction mixture represented high DPPH radical scavenging activity. The inhibition of DPPH radical scavenging activity in percent was calculated by the following equation:

$$\text{Inhibition (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (2)$$

where A_c = absorbance measured without sample and A_s = absorbance measured in the presence of samples

2.8 Statistical analysis

Statistical evaluation was performed using SPSS software version 17.0 (Chicago, USA). Results are presented as mean±standard deviation. The one way ANOVA test was used for statistical comparison, where $P<0.05$ was significantly considered. All data represent at least 3 independent experiments.

3. Results

3.1 Visual observation and UV-visible spectroscopy

In this study, the AgNPs were synthesized from $AgNO_3$ (1 mM) using different concentrations of *G. glabra* root extract as the reducing agents, and a microwave as the reaction accelerator. After heating for 2 min, the color of all mixtures changed from yellowish brown to colloidal brown (Figure 1). Compared to the pattern of the mixture before heating, the absorbance spectrum of the mixture (concentration ratio at 1:4) after heating showed a specific pattern at ~430 nm which indicated the formation of AgNPs (Figure 2). Plasmon resonance band spectra of all mixture ratios also displayed specific peaks at a similar wavelength. The absorbance spectra increased which corresponded to the concentration of the extract in the mixtures (Figure 3). All mixture ratios that gave an absorbance peak at 430 nm (ratios of 1:0.5 to 1:4) were selected for analysis in the next steps.

Figure 1. Synthesis of AgNPs using various concentration ratios of $AgNO_3$ (mM) and *G. glabra* root extract (mg/ml) as 1:0.1, 1:0.5, 1:1, 1:2, 1:3, and 1:4. The image shows solutions after heating for 2 min with color changes upon formation of the AgNPs.

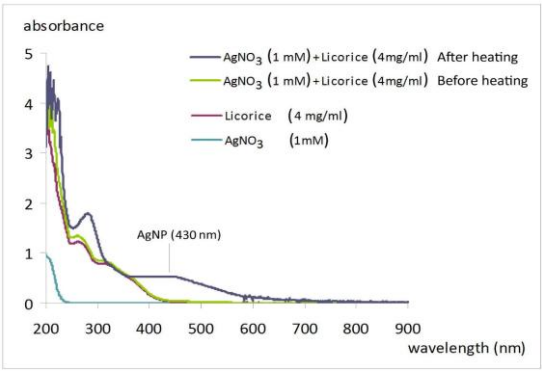

Figure 2. UV-visible spectra of $AgNO_3$ + licorice (*G. glabra*) root extract solutions before (green) and after (dark blue) heating for 2 min. The image shows a specific absorbance pattern (~430 nm) of the solution after heating which indicated the appearance of AgNPs. The spectra of licorice extract (brown) and $AgNO_3$ (light blue) were also included as controls. The absorbance pattern of the controls did not change either before or after heating (data not shown).

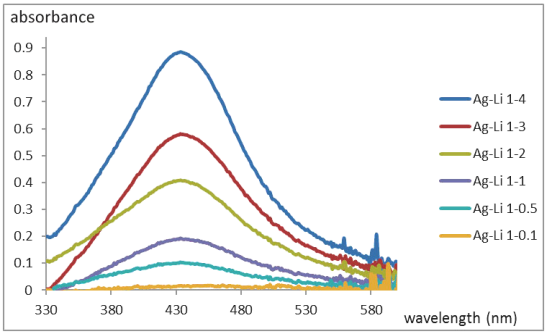

Figure 3. UV-visible absorbance peaks of the AgNPs synthesized using various concentration ratios of $AgNO_3$ (mM) and licorice (*G. glabra*) root extract (mg/ml) as 1:0.1, 1:0.5, 1:1, 1:2, 1:3, and 1:4. The image shows surface plasmon resonance peaks of all mixtures at 430 nm. The peaks were calculated by the subtraction of all spectra between the patterns after and before heating for 2 min.

3.2 Characterization of synthesized AgNPs

The SEM images at a magnification of 10,000x revealed small grains of AgNPs in spherical shapes (Figure 4). All selected mixture ratios gave similar images (data not shown). The sizes and shapes were measured in details by TEM images, in which most of the AgNPs produced in all mixtures exhibited as a similar round shape (at magnification of 310,000x) with average sizes of ~20 nm (range 20-30 nm) (Figure 5). The surface roughness and topography of the AgNPs were determined using AFM. The AFM images with 2 µm resolution showed no agglomeration and also confirmed the spherical shape of the AgNPs (Figure 6).

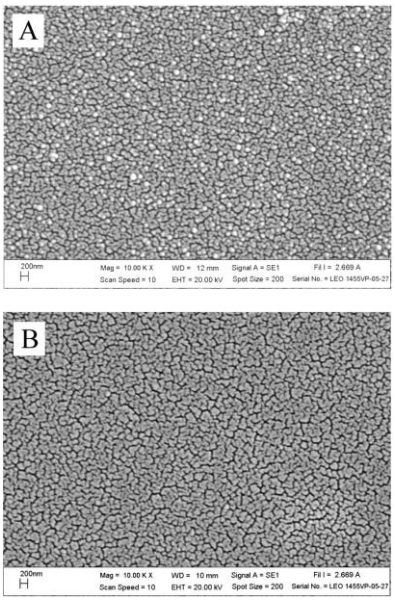

Figure 4. SEM image shows small grains of synthesized AgNPs on coated primer PDADMAC/PSS (A), compared to an image of the control sample without AgNPs (B). The AgNPs synthesized using various concentration ratios of $AgNO_3$ (mM) and *G. glabra* root extract (mg/ml) gave similar SEM images. This representative image visualizes AgNPs synthesized from the ratio 1:2.

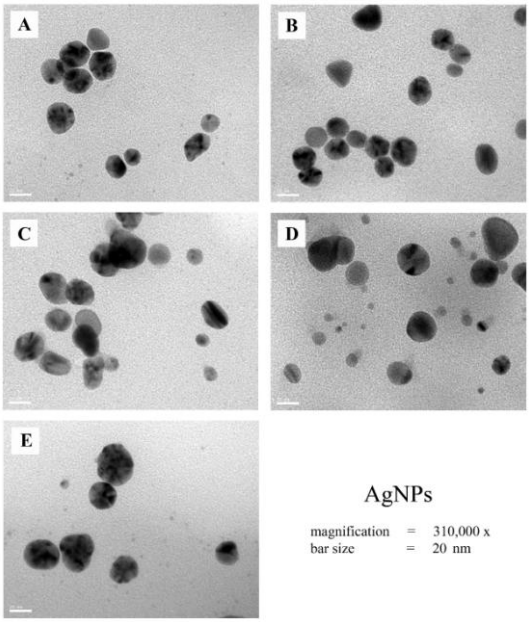

Figure 5. TEM images show AgNPs synthesized using various concentration ratios of $AgNO_3$ (mM) and *G. glabra* root extract (mg/ml).
(A) 1:0.5 (average size of AgNP=22.13±1.67nm.)
(B) 1:1 (average size of AgNP= 22.13±0.46nm.)
(C) 1:2 (average size of AgNP =26.93± 0.62nm.)
(D) 1:3 (average size of AgNP=24± 0.8nm.)
(E) 1:4 (average size of AgNP=28.27± 4.03nm.)

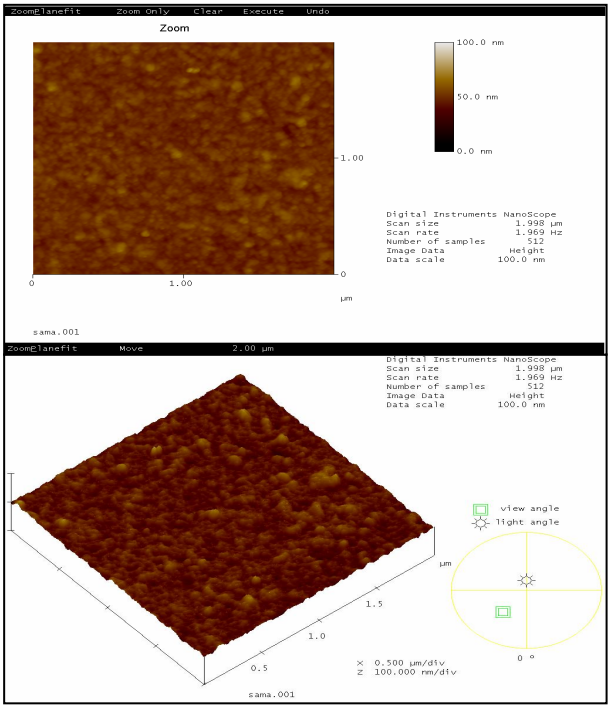

Figure 6. AFM analysis with 2x2 µm resolution revealed topography of synthesized AgNPs. The images display the surface roughness of a 2x2 µm scanning area, and a 3-dimensional field micrograph showing the surface of spherical particles without agglomeration.

3.3 Antimicrobial property of the AgNPs

All synthesized AgNPs in the mixtures showed inhibition zones against *S. mutans* when examined by agar well diffusion assay (Table 1). The antimicrobial activity tended to increase as the concentration of the extract in the mixture decreased. However, due to the instability of the nanoparticles that appeared at concentration ratios higher than 1:2 (data not shown), the mixture ratio of 1:2 was chosen. By MIC and MBC assays, the AgNPs in the extracts exhibited more bactericidal activity than the $AgNO_3$ or *G. glabra* extract alone. The MIC and MBC of AgNPs were 6.25 and 25 µg/ml, respectively (Table 2).

Table 1. Antimicrobial activity of synthesized AgNPs against *S. mutans*. Each value represents an average of 3 separately conducted experiments.

Streptococcus mutans	Inhibition zone (mm) (mean±SD)
AgNPs 1:0.5 ($AgNO_3$: *G. glabra extract*)	11.33±0.02
AgNPs 1:1 ($AgNO_3$: *G. glabra extract*)	9.60±0.14
AgNPs 1:2 ($AgNO_3$: *G. glabra extract*)	10.00±0.39
AgNPs 1:3 ($AgNO_3$: *G. glabra extract*)	10.67±0.47
AgNPs 1:4 ($AgNO_3$: *G. glabra extract*)	9.00±0.05
$AgNO_3$	8.93±0.04
0.2% Chlorhexidinegluconate	17.00±0.05
G. glabra extract	6.95±0.01

Table 2. MIC and MBC of synthesized AgNPs against *Streptococcus mutans*

Strain	MIC (µg/ml)		MBC (µg/ml)	
	G. glabra extract	AgNPs	*G. glabra extract*	AgNPs
Streptococcus mutans	780	6.25	1560	25

3.4 Effect of the AgNPs on head and neck squamous carcinoma cell line HN-30

At a concentration of 10 µg/ml, the AgNPs showed 70-80% cytotoxicity to HN-30, but indicated no cytotoxicity on the HGF (Figure 7). A positive correlation between AgNPs and cytotoxicity rate was observed. *G. glabra* extract itself did not exhibit any cytotoxicity to either the HGF or HN-30, even at a high concentration of the extract (100 µg/ml) and viability of 95% was detected in both cell lines (Figure 7).

3.5 Free radical scavenging activity of AgNPs

The color of all reactions was found to change from purple to yellow, thus indicating free radical scavenging activity. All concentrations of synthesized AgNPs showed obvious inhibition of DPPH activity at approximately 60-80% compared with ascorbic acid. This seemed to be in a dose-dependent manner (Figure 8).

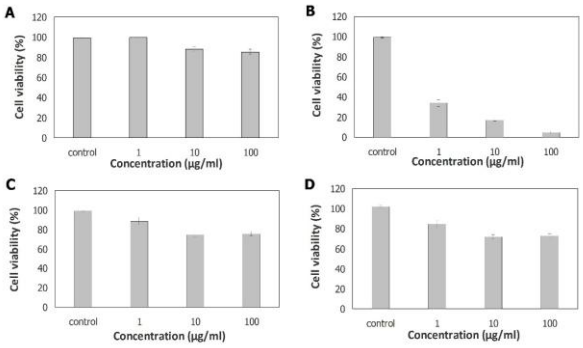

Figure 7. Effects of AgNPs are shown with various concentration ratios on viability of human gingival fibroblasts (HGF) (A) and head & neck cancer cells (HN-30) (B), as well as the effects of *G. glabra* root extract alone on the viability of HGF (C) and HN-30 (D). All cell types were incubated with AgNPs or the extracts for 24 h before testing by MTT assay. Cell viabilities are calculated as percentages compared to controls (untreated groups), and presented as average values with standard deviations (error bars). The statistical significance ($P<0.01$) was evaluated by one-way ANOVA. All measurements were performed in triplicate.

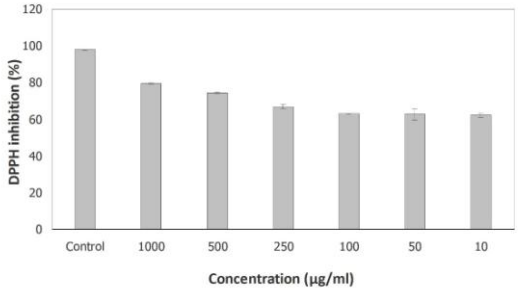

Figure 8. Activity of synthesized AgNPs as anti-oxidants was determined using the DPPH inhibition assay. Ascorbic acid (10 µg/ml) was used as the control sample. Each value represents an average of 3 separately conducted experiments.

4. Discussion

The method for rapid and green synthesis of silver nanoparticles in this study was developed using *G. glabra* root extract as a biomaterial to assist in the production of AgNPs in an eco-friendly way. Sreelakahmy *et al*. (2016) and Dinesh *et al.* (2012) also chose *G. glabra* root extract as the reducing biomaterial. Both researchers synthesized AgNPs from $AgNO_3$ within 7 h and 30 min. For faster production, we applied microwaves to speed up the reaction to produce the AgNPs in 2 min. The result of the UV-visible spectra showed an absorbance peak at 430 nm. UV-visible spectroscopy is an important tool to confirm the formation and the stability of metal nanoparticles in aqueous solutions (Krebig & Vollmer, 1995). The change in the color of the solution from yellowish to reddish brown was due to the excitation of surface plasmon resonance in the AgNPs (Obaid *et al*., 2015; Veerasamy *et al.*, 2011). Absorbance of AgNPs is generally found in the visible range of 420-440 nm (Yakout & Mostafa, 2015). Our results

clearly indicated the formation of AgNPs with an absorbance at 430 nm. The synthesized AgNPs were visualized as small round grains in the SEM images, whereas the TEM images showed more details of each particle as a spherical form with an average size of ~20 nm. A 3D micrograph from an AFM analysis also supported the round shape of our AgNPs without any agglomeration. AgNPs synthesized using various concentration ratios of $AgNO_3$ and *G. glabra* root extract gave similar morphologies.

Our synthesized AgNPs exhibited antimicrobial activity against *S. mutans* which is the important causative agent in oral disease. A similar study performed on *S. mutans* biofilm found that AgNPs at a concentration >100 ppm reduced the formation of biofilm (2.3 log) (Pérez-Díaz *et al*., 2015). Although the production processes or the capping agents were different in other studies, the antimicrobial activities were also reported to kill many other pathogens, such as *H. pylori*, *Salmonella sp.*, *Staphylococcus aureus*, and *Bacillus cereus* (Castañón *et al*., 2008; Ghaffari-Moghaddam & Hadi-Dabanlou, 2014; Lalitha *et al*., 2013; Martínez-Nabikhan *et al*., 2010; Pourmortazavi *et al*., 2015; Sasikala *et al*., 2015; Veerasamy *et al*., 2011). An insight behind the killing mechanism is probably due to the release of Ag^+ from the AgNPs. AgNPs can anchor onto the cell wall of bacteria to produce pits and slowly release Ag^+ into the cytoplasm (Ahmed *et al*., 2016; Prabhu & Poulose, 2012; Pourmortazavi *et al*., 2015). Free radicals of reactive oxygen species (ROS) induced by metal ions can damage bacterial membranes, mitocondria, and DNA which results in oxidative stress and finally cell death (Ahmad & Sharma, 2012; Ahmed *et al*., 2016; Goodarzi *et al*., 2014; Lalitha *et al*., 2013; Prabhu & Poulose, 2012). With the assistance of bio-organic compounds in the extract, several natural ligands, such as saponin, tannin, terpenoids, and flavonoids, in the *G. glabra* extract can interact with the microbial membrane (Dinesh, 2012; Goodarzi *et al*., 2014). They possibly helped AgNPs in the killing of *S. mutans* in our study.

Head and neck cancer is the most frequent type of cancer in the oral cavity which accounts for approximately 10% of the total number of cancer cases in Thai males (Tanadech, 2011). In this study, AgNPs in the extract (10 µg/ml) was able to kill nearly 80% of human head and neck carcinoma cells, but had no effect to HGF, thus indicating a potential in oral disease therapy. The cytotoxic effect may be caused by the active physicochemical interaction of silver atoms with functional groups of intracellular proteins as well as with nitrogenous bases or phosphate groups in the DNA (Miura and Shinohara, 2009; Han *et al*., 2014). Cytotoxicity on breast cancer cell line MDA-MB-231 was explained by inhibition of cell growth and lactate dehydrogenase activity (Gurunathan *et al*., 2003), an increase of ROS (Hsin *et al*., 2008), and generation and activation of caspase-3 (Han *et al*., 2014). These lead to cell apoptosis and death (Hsin *et al*., 2008; Miura & Shinohara, 2009; Moaddab *et al*., 2011). Although, the reason to explain the low cytotoxic effect to HGF is unknown, a similar mechanism might occur.

In addition, our biosynthesized AgNPs possessed free radical scavenging activity. This could be a property of the *G. glabra* extract. The activity and stability of the AgNPs are affected by plant extracts (Ahmed *et al*., 2016; Banerjee *et al*., 2014; Dinesh *et al*., 2012; Ghaffari-Moghaddam & Hadi-Dabanlou, 2014; Goodarzi *et al*., 2014; Pourmortazavi *et al*., 2015; Prabhu & Poulose, 2012). The phenolic compounds in the extracts are strong antioxidants. They can behave as an electron donor and singlet oxygen quenchers (Ahmad & Sharma, 2012; Goodarzi *et al*., 2014; Lalitha *et al*., 2013;). However, the effects of AgNPs as antioxidants are still not elucidated.

5. Conclusions

We synthesized AgNPs by a rapid biological process. *G. glabra* root extract was chosen as the reducing agent to generate the AgNPs. The microwaves accelerated the process and the AgNPs were achieved within 2 min. In this study, AgNPs formed into a spherical shape with sizes that ranged from 20 to 30 nm. They were monodispersed and no agglomeration occurred. Our AgNPs indicated antimicrobial activity against *S. mutans* and possessed free radical scavenging activity. They exhibited cytotoxicity to human head and neck cancer cells, but were harmless to human gingival fibroblasts. Our results suggested that the *G. glabra* root extract-capping AgNPs have the potential for application in the dental and biomedical fields. However, further studies in other aspects and applications are still required.

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