RESEARCH PAPER

Nanotoxicity for E. Coli and Characterization of Silver Quantum Dots Produced by Biosynthesis with Eichhornia Crassipes

Angelica Silva[1], Sonia Martínez-Gallegos[1]*, Genoveva Rosano-Ortega[2], Pablo Schabes-Retchkiman[3], Carlos Vega-Lebrún[2] and Veronica Albiter[1]

[1] *División de Estudios de Posgrado, Instituto Tecnológico de Toluca Av. Tecnológico S/N Ex Rancho La Virgen, Metepec, México, C.P. 52140*

[2] *Universidad Popular Autónoma del Estado de Puebla (UPAEP). 17 sur no. 901 Barrio de Santiago, Puebla, Puebla, México. C.P. 72410*

[3] *Instituto de Física, Universidad Nacional Autónoma de México, Apartado Postal 20-364, Distrito Federal., México.*

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ABSTRACT

Nanomaterials are widely used in health and biomedical applications, however, only a few studies investigate their toxic effects. The present report signifies a contribution to the study of the toxic effects of silver nanoparticles on E. coli cells, which is a model organism of anthropogenic pollution. The toxicity of nanoparticles depends on their chemical and surface properties, shape and size. Nanoparticles that have the same chemical composition but different shapes or sizes might have different effects on cells. In this work, Ag nanoparticles were biosynthesized with an Eichhornia crassipes biomass, and it was demonstrated for the first time, that the amounts of hydrolysable tannins in this plant, are directly related to the size, shape, structure and composition of the Ag nanoparticles ; furthermore, the toxic effect was studied using E. coli cell culture. The EC was divided in three sections, i.e. roots, stems and leaves. Particle aggregation seems to be influenced by the amount of tannins present in the biomass. For each plant part, the amounts of hydrolysable tannins were determined, the highest amounts of these chemicals were present in the leaves, and hence these Ag nanoparticles dissolutions were used for the nanotoxicity experiments. . The cytotoxicity of Ag nanoparticles in a suspension was tested using the Ag nanoparticles synthesized with leaves, against Escherichia Coli ATCC 25992 where the concentration that inhibited 100% of bacterial growth, was 5 mg/L in contrast with a commercial solution which needed 10mg/L of Ag. For the most part, the Ag nanoparticles seemed to be of a nearly spherical shape, although on closer examination were determined to be mainly polyhedral. Leaves biomass, produced mainly quantum dot nanoparticles with sizes below 10 nm and the Ag nanoparticles were mostly AgO. The cytotoxicity of Ag NPs in a suspension tested using the Ag nanoparticles on E. coli was highly effective towards inhibition of bacterial growth.

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INTRODUCTION

There has been a lot of recent interest in the synthesis of metallic nanoparticles, especially silver and gold because of their industrial and agricultural applications, consumer products and medical-hospitalarian uses, especially for diseases like cancer, diabetes, arthritis, hepatitis, tuberculosis, etc., due to their electrical and

* *Corresponding Author Email: Soniazteca@hotmail.com*

physicochemical properties, making it necessary to facilitate their synthesis [1, 2].

In particular Silver nanoparticles (AgNPs) have applications in optics, sensors, biologic image formation, catalysis, anti-cancer treatments and water disinfection [2, 3]. AgNPs detection in suspension is relatively easy, since a UV-Vis scan shows an absorption peak corresponding to a surface plasmon available only when the metallic particles are present between 420-430 nm [3].

On the other hand, a special effort has been dedicated to finding new methods, environmentally friendly, clean, simple and at low cost with a good enough yield of NPs [2-4]. In choosing a synthesis method one should consider the size, shape and composition desired for the NP application, the methods involving chemical agents, which can be dangerous or toxic, are the ones that up to the present guaranteed such control. Some of the methods used are thermal decomposition, electrochemical, microwave and recently biosynthesis [5].

The use of biomaterials or green materials, rich in tannins for the biosynthesis, offers an environmentally friendly alternative; since they are reductive agents by nature and make it possible to obtain metallic nanoparticles [6, 7]. The use of natural materials has been reported in the literature, that have succeeded in obtaining Ag or Au NPs, for instance Bamboo [4]; Ceropegia thwaitesii [7], Black tea [6] ; powders from Solanum tricobatum, syzygium cumini, centella asiática and Citrus sinesis [5] ; Amaranthus dubius, amaranthus polygonoides, Alternantheras essilis, Portula caoleracea, Pisonia grandis, Kedrostis foetidissima [8] and Eichhornia crassipes [9] among others. In the reports cited, the amounts of hydrolysable tannins are not related to the shape, size and chemical composition of the AgNPs.

In the present work the synthesis of AgNP is reported using Eichhornia crassipes, divided in three main sections (leaves, stem and roots), and the influence of each plant section is studied through the amount of hydrolysable tannins contained in each plant part in the synthesis of AgNPs.

Eichhornia crassipes (EC) or water hyacinth is a macrophyte generally considered as an aquatic weed in the bodies of water where it is found, for some people it is considered one of the worst plant pest because when the water body is not controlled, the hyacinth can cover it completely, blocking the light passage to the interior and negatively affecting the flora and fauna of the given infested place and it becomes difficult to eliminate. Water hyacinth is a green plant that contains a great deal of metabolites like for instance, phenolic compounds, aromatic compounds, flavonoids, phenols, etc. [9].

Tannins are plant polyphenolic compounds located in various parts of plants, such as wood, bark, fruit peels, pods, leaves, roots and plant galls. They are also powerful antioxidants (reductants) capable of eliminating reactive unpaired electrons in oxygen radicals by delivery of electrons which are in sufficient quantities in many hydroxyl groups. Tannins are generally divided into hydrolysable tannins (HT) and condensed tannins (CT). For the HT the monomer units are gallic and ellagic acid, this substance is part of pharmacopoeias since the middle of last century and has proven antibacterial effects, but it is also suspected of hepatotoxicity and cytotoxicity [3].

Microbacterial activity of AgNPs and cytotoxicity in biosynthesized particles has been measured through several models [4-9], in particular Escherichia coli (E. Coli) has been used frequently.

E.coli is a common bacteria in the gastrointestinal tract, and part of the normal bacterial flora. However, some E.coli strains are able to produce a toxin that could produce serious infection. The main reservoir of such E.coli strains is contamination by fecal matter due to poor processing methods which might end up contaminating other foods (e.g. milk, vegetables) and water. [10]

The central idea of this paper consists in determining the effect of biosynthesis of AgNPs with EC and their cytotoxicity effects on the model bacteria E. coli. To accomplish this goal the EC was divided in three different sections, with the desire to determine HT amount present in each part of the plant, analyze the influence of tannins present on the formation of AgNPs synthesized as a function of the pH of the solution, their size, shape and chemical composition and to determine the best preparation conditions for quantum dots.

MATERIALS AND METHODS

Eichhornia crassipes sampling

Approximately $2m^2$ were collected from hyacinth adult plants Eichhornia crassipes, from the Chignahuapan lake located in Almoloya del Río, State of Mexico, Mexico. The sampling was done according to the NMX-AA-014-1980 standard [10], storing and transporting the plants in polyethylene containers. The collected samples were washed

several times with running water, and then dried at room temperature for 15 days. Once dry, the plants were separated in three parts: leaves, stem and roots; each section was independently ground in an industrial liquefier (OSTER) and strained through 20 mesh, then washed twice with a water and HCl solution (Sigma-Aldrich) 0.01N, with 30 minutes rest between washes and finally dried in an oven (Riossa) at 50 °C for 48 hours [11].

Biomass characterization

Polyphenol Identification in each section of Eichhornia crassipes plant

15 mg of the dried biomass (roots, stems or leaves) of Eichhornia crassipes were placed in a polyethylene tubes, then 30 mL of an ethanol-water solution (J.T.Baker) (70:30v/v) were added and left in contact for 15 days with continuous vigorous agitation. Finally, the samples were filtered and a spectrophotometer scan UV-Vis (Perkin Elmer lambda 35) was performed at 200-800 nm of wavelength , using as a blank deionized water [12].

Total Phenol quantification by section of Eichhornia crassipes

The total phenol quantification was realized according to NMX-AA-050-SCFI-2001 [13]. Samples of 0.25 mL were mixed with 2.25 mL of ethanol/water (70:30 v/v) for study with a spectrophotometer UV-Vis at 510 nm. A calibration curve was obtained with a phenol solution (Sigma Aldrich) from 0 to 10 mg/L.

Hydrolysable Tannin (HT) quantification by Eichhornia crassipes section

Extracts preparation

For each section of the Eichhornia crassipes plant, 10 mg of the biomass was weighed, each part of the plant section was then put in contact with a 5 mL solution of i-PrOH (Aldrich)-water (65:35), and then the mix was agitated for 20 minutes. This procedure was repeated three additional times, so the ending mixture i-PrOH-water-biomass volume was 20 mL and the ending concentration was 500 mg/L [14].

HT Hydrolisis

5 mg of the biomass were placed in a tube and mixed with 10 mL of the i-PrOH-HCl (65:35) solution. The mixture was left to rest for 24 hours and filtered. In a separate tube 1.5 mL of the extract was placed; 1.55 mL of HCl 12 N and 1.45 mL of i-PrOH, were added, placed in a thermal bath at 90°C for 180 min, at the end a spectrophotometer scan UV-Vis (200- 800 nm) was performed to ascertain that the hydrolysis was successful by ellagic acid detection at 368 nm, as a blank i-PrOH-HCl (65:35) was used [14].

Calibration curve for HT

This was determined using aliquots of 1, 5, 10, 20, 30 y 50 mg/L of ellagic acid., prepared from a solution of the same acid of 50 mg/L for which a solvent i-PrOH-HCl (65:35) was used. The samples were scanned by spectrophotometer UV-Vis at 368 nm utilizing as a blank i-PrOH-HCl (65:35).

HT Quantification

0.25 mL of the obtained solution in the hydrolysis of tannins, were mixed with 2.25 mL i- PrOH-HCl (65:35). The solutions were analyzed in the Spectrophotomer UV-Vis at a wavelength of 368 nm. To get rid of interferences due to flavonoids and chlorophylls, as a blank the first extract 0.25 mL were mixed with 2.5 mL of i-PrOH-water (65:35). The values were interpolated from the calibration curve of ellagic acid and multiplied by a gravimetric factor of 2.9 for their estimate as HT. This factor was established taking into account the mean molecular mass (MR 1867 g/mol) between casuarictin (monomer), nobotanin B (dimmer) and nobotanin E (trimmer). Each one of those ellagitannins liberates two units of ellagic acid with MR 322 g/mol). The gravimetric factor was calculated according to Isaza JH, et al [14].

$$Gravimetric\ Factor = \frac{1867 g/mol}{2x322 g/mol} = 2.9$$

Ag NPs Synthesis

The biomasses of Eichhornia crassipes (roots, stem and leaves) were washed in deionized water, then agitated in an ultrasonic bath (Branson® CPX3800H) centrifuged (3000 rpm, 20 min), and mixed with a buffer solution (Sigma Aldrich) to adjust for pH 4, 7, 10 and 12, to get NPs at different pH values, before being agitated again. Finally 25 mL of $AgNO_3$ 0.003 M was added, the mixture was agitated again and left to rest. The excess biomass were separated by filtering.

Nanoparticle Characterization

HRTEM, TEM and EDS Analysis

The samples were prepared for HRTEM, TEM and EDS analysis, as follows, a droplet was extracted from each nanoparticle sample solution

prepared (root, stem or leaves) for a given pH; it was then placed on a carbon coated 3mm electron microscope carbon coated grid and left to dry in vacuum. All the TEM, HRTEM, EDS analysis were performed in a JEOL JEM-2010F FasTem microscope equipped with a field emission gun, with a 0.2 nm resolution at the Physics Institute, UNAM. The results obtained were the shapes, sizes, structure and phase distribution of the AgNP; EDS was done for global and individual sample elemental composition.

Separation of AgNPs

Two biosynthesized dissolutions were used, the first as obtained after the procedure (including the larger particles >10nm in size) at pH12 according to the bioreduction method and the second after decanting from the original dissolution the sediment obtained, after centrifuging at 10,000 rpm for 40 min according to the paper by Galazzi et al. [15]. After, the sediment containig the large AgNPs was dehidrated at 40°C/4 hours. The powder was used to prepare the second dissolution of AgNPs in the range of 2-100 mg/L of Ag.

Cytotoxicity antibacterial assay

For antibacterial activities of the compounds, wells were made in plates containing as a model strains of E. coli ATCC 25992. The minimum bactericidal concentration (MIB), was defined as the mínimum amount of AgNPs in mg/L required to inhibit growth of 100% of the microorganisms presenr in the initial count of the E. coli inoculum (from 400 to 850 CFU/mL).

Cytotoxicity was evaluated comparing the efficiency of four Ag containing dissolutions: 1) Commercial ionized Ag dissolution; 2) A $AgNO_3$ solution; 3) Bioreduced AgNPs as prepared and 4) Dissolution prepared from the powder. As a control and reference a solution prepared with just the EC biomass without metal salts added was used.

RESULTS AND DISCUSSION

UV Spectroscopy Results

The results of the detection by UV spectroscopy of polyphenols in roots, stems and leaves of Eichhornia crassipes, are shown in Fig. 1. In every sample the absorption maxima in the range 277-280 nm, were observed. These peaks are associated with the presence of Polyphenols in the structure of the plant, which in turn are responsible for the antioxidant activity and act as potential reducing agent due to the OH groups in their structure. This behavior is similar to that reported for other green plants [4, 12, 16-19]. Plants like Eichhornia crassipes, have a tendency to accumulate excessive amounts of Polyphenols as a defense and adaptation strategy, due to the stress they are exposed, as a result of living in the water and the physico-chemical characteristics of the bodies of water where they are found [12, 20].

The total Phenols determination, was performed in the Eichhornia crassipes sections, before and after an acid wash; the results are shown in Table 1. The HCl 0.01N reduces the concentration of phenols in the three sections of the plant. The biggest loss of phenol compound concentration occurs in the roots. This can be attributed to the fact that this section constitutes the first barrier to the collection and translocation of substances when in direct contact with the environment, and this is the reason of the susceptibility for solubilization in acid conditions, on the other hand it has been reported that this same section is capable of removing a greater amount of contaminants in the water environment [11, 20, 21].

In contrast, a loss of phenols was observed in the leaves and stems, although less in the leaves than in the stems. This might be due to the fact that in both these sections Chlorophyll is detected, which in turn favors the synthesis of metabolites including phenolic substances, which are detected at 278 nm, this justifies the peak being more intense in these two (green) sections,. The detection and quantification of total phenols,

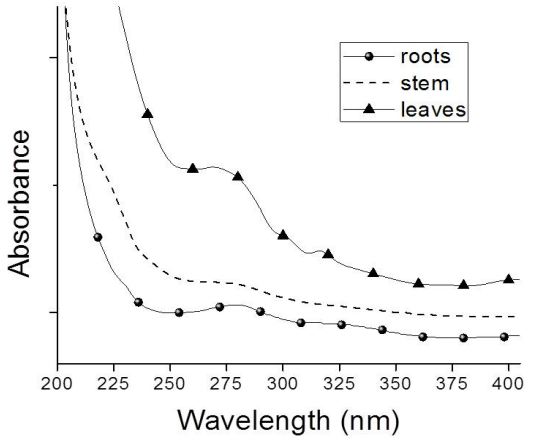

Fig. 1. UV spectra for polyphenols in roots, stem and leave of Eichhornia crassipes.

Table 1.Total phenol in mg/L in Eichhornia crassipes section with or without acid wash (R=roots, S=steam, L=leaves)

Treatment	Section	mg/L
Without acid wash	R	7.4 ± 0.53
	S	5.6 ± 0.20
	L	8.2 ± 0.95
With acid wash	R	3.9 ± 0.51
	S	4.2 ± 0.42
	L	6.6 ± 1.17

shows that Hyacinth contains in its structure phenol compounds including: simple phenols, cumarins, lignins, lignans, condensed and hydrolysable tannins, phenolic acids and flavonoids. All of them connected to the antioxidant activity of the plant that works as a natural defense mechanism [22]. These results are in line with those reported in the literature, where it has been mentioned that the highest amount of aromatic alcohol phenols are found in the most exposed parts of the plants, such as leaves, moreover, their structure makes them highly resistant to chemical degradation and this is why the acid wash is not capable enough of solubilizing the phenol structures detected in the Eichhornia crassipes sections [23].

The experimental conditions realized in the present work, applied to Eichhornia crassipes, produce the hydrolysis of nobotanin B, which is responsible for the formation of the Ellagic acid formed by hydrolysable tannins, detected by UV-Vis at 368 nm [14]. The hydrolysis of the different sections of Eichhornia crassipes, shown in Fig. 2, displays two absorption peaks, one at 368 nm characteristic of the Ellagic Acid molecule and another at 280 nm that identifies the polyphenols present in the hydrolyzed substance. The fact that the ellagic acid peak is detected, confirms the

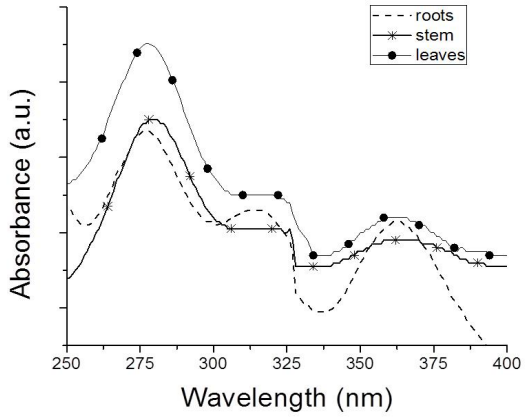

Fig. 2. UV spectra for ellagic acid in roots, stems and leaves of Eichhornia crassipes.

presence of hydrolysable tannins in the plant used in this study, and hence it is possible to quantify it for each of the sections of the water hyacinth and relate it to the Ag nanoparticle production.

The quantification of the hydrolysable tannins (ellagitannins) is shown in Table 2. The HT concentration amongst sections grows, according to the degree of the plant exposure, the leaves showed the highest concentration of hydrolysable tannins measured as Ellagic Acid.; this confirms that the main difference between the plant sections can be attributed to the tannins becoming a defense mechanism of the plants, and with the leaves more palatable for herbivores, the tannin production increases [24].

During the nanoparticle synthesis, the starting Ag nitrate solution was of a yellowish color, after the pH adjustment, it became Brown-yellowish; the presence of NP is detected by the brown color of the solution, which is consistent with the reports in the Literature for the formation of Ag NPs [25-27]. The presence of polyphenols in the AgNP suspensions is associated to the reduction and stabilizing effect of the phenolic compounds and HT of the synthesis procedure.As reducing agents of the silver ions, and stabilizers they achieve production of organic molecules which in turn are adsorbed on the surface of the NPs preventing them from agglomeration [11, 28, 29].

Electron Microscopy Results

Electron microscopy images were obtained from leaves, stems and roots at different pH. In all cases AgNPs were detected. In Fig. 3 we show micrographs for leaves as the biomass, showing that for the many tested pH's in particular pH4, pH7, pH10 and pH12 AgNPs were formed. At pH12 the smaller particles with best distribution were produced.

Fig. 4 shows an EDS analysis of the AgNP obtained with Hyacinth leaves as expected the main constituent is Ag confirming that the NPs are built up of this element mainly. Other elements detected were C, O, P. (The Cu signal comes from the sample grid and holder).

As shown in Fig. 5 all plant sections produced nanoparticles with sizes <10nm, however, the pretreatment of roots is complex because it carries sediments and can contaminate the samples. In this Fig. it is clearly shown that AgNps created by leaves biomass are smaller and have higher density than roots, which contain smaller amounts of HT.

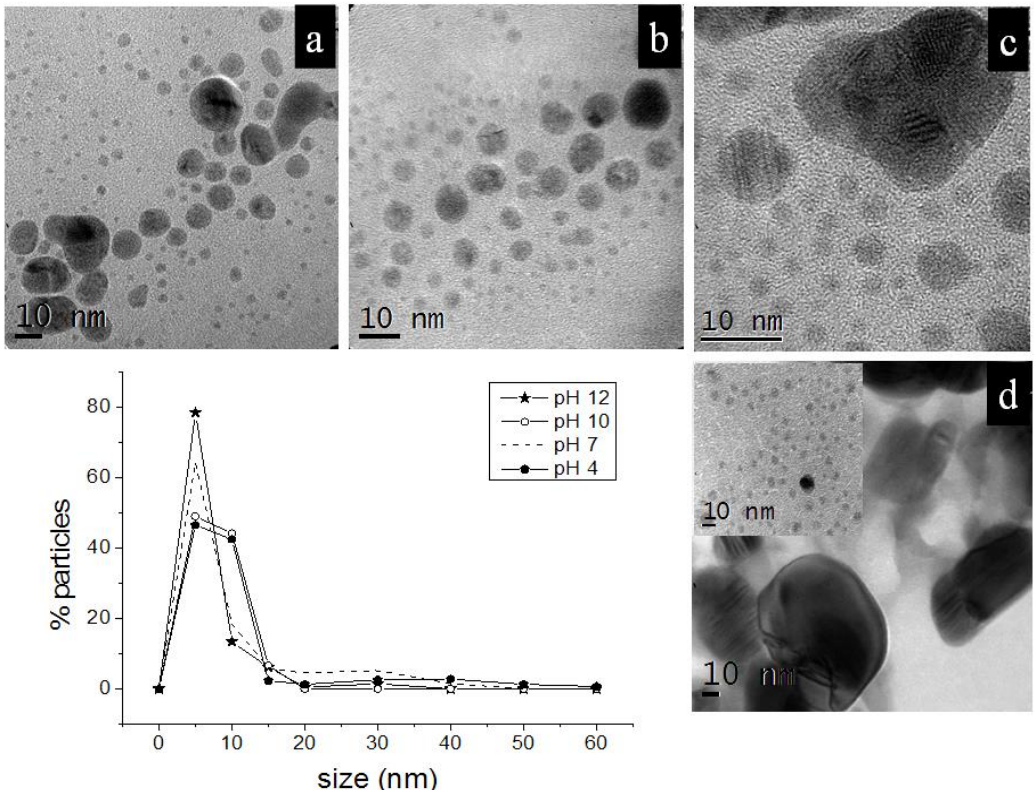

Fig. 3. Size distribution analysis of the produced nanoparticles for the different pH conditions for leaves. TEM images for (a) pH = 12, (b) pH = 10, (c) pH= 7 and (d) pH = 4 with the corresponding (e) size distribution plot.

Table 2. Hydrolysable tannins determined as ellagic acid (E.A.) and hydrolysable tannins (HT)

Eichhornia	E.A (mg/L)	% E.A.	% HT
Roots	40.45 ± 5.6	8.1± 1.1	23.5 ± 3.3
Stem	50.76 ± 10.1	10.2 ± 2.0	29.4 ± 5.8
Leaves	61.97 ± 10.1	12.4 ± 2.0	35.9 ± 5.8

In Fig. 6 a composite of HRTEM micrographs as a function of pH is shown for AgNPs obtained from leaves biomass. All particles were identified as either Ag or AgO. In Fig. 6a and 6c all polyhedral particles identified, from the FFT (inset), were identified and characterized as AgO or Ag particles; in Figs. 6b and 6d only particles of AgO were found. The small arrows in the Fig. 6 show the presence of defects such as dislocations, stacking faults and twins. In Table 3 a compilation of the HRTEM characterization is shown, regarding shape, size chemical phase and structure for the different pH values for leaves.

Although in the bioreduction process we expect to fully reduce the Ag phase to Ag^0, water molecules, phenolic compound can help produce a silver oxide in their reduction process, as proposed in equations 1-3.

$$AgNO_3 \xrightarrow{H_2O} Ag^+ + NO_3^- \quad (1)$$

$$Ag^+ + H_2O \rightarrow AgOH + H^+ \quad (2)$$

$$Ag^+ + H_2O \rightarrow AgOH + H^+ \quad (3)$$

On the other hand, for acidic conditions (acidic pH) the synthesis promoted only the formation of AgO because of the H^+ excess. Under basic conditions, Ag and AgO NPs were obtained with final composition affected by the high content of OH. Neutral pH promoted Ag, AgO because of H^+ and OH^- being in equilibrium.

In general biosynthesis by using leaves of the EC as biomass, showed the formation of stable Ag NPs as observed by TEM.

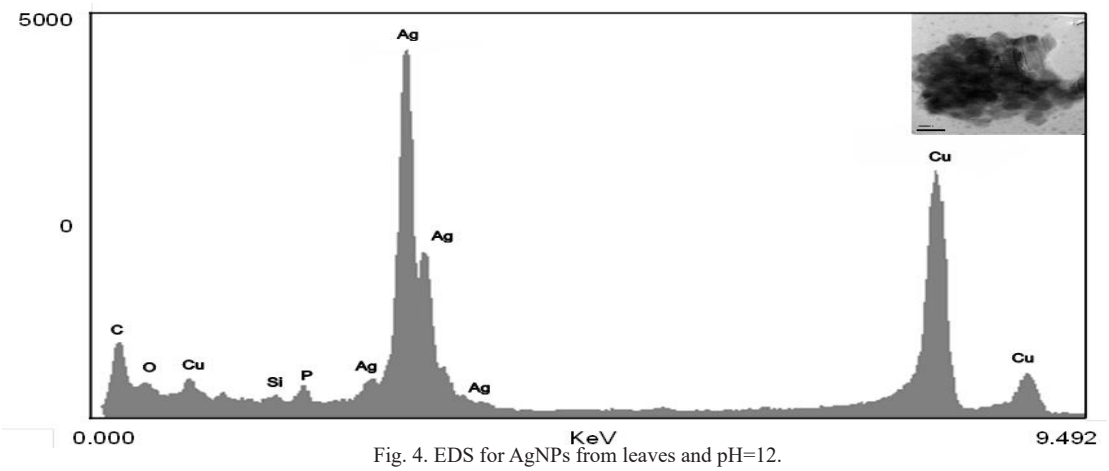

Fig. 4. EDS for AgNPs from leaves and pH=12.

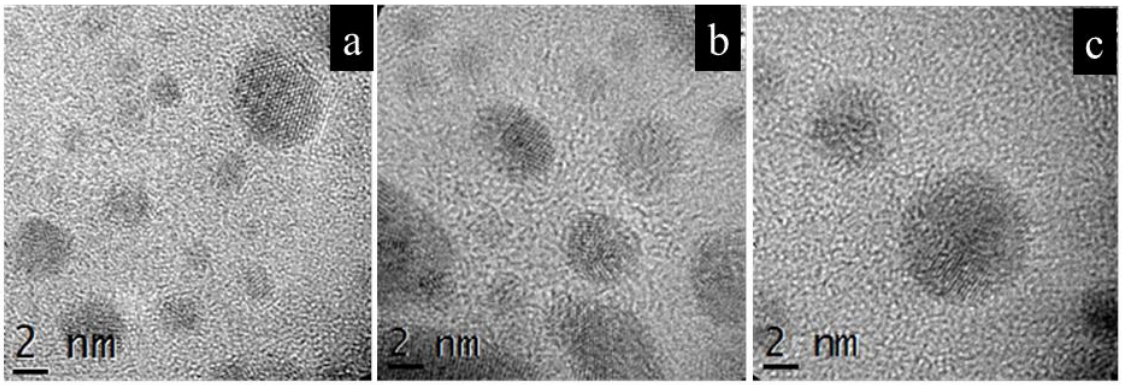

Fig. 5. Ag Nanoparticles for the different plant sections at pH10, (a) leaves, (b) stems and (c) roots

Extraction of the AgNPs

According to the characterization results, the amount of HT in each section of the plant, influences clearly the synthesis of the nanoparticles. The higher HT was detected in the leaves; this section of the plant promotes the formation of Ag NPs of a polyhedral profile in the most, and the majority of them were smaller than 10 nm without aggregation; for this reason, we use the suspensions prepared with this plant section.

A Ag nanoparticle powder was obtained from the dissolution. The best extraction of AgNP was for the pH12 and pH10 samples by the decanting method.

In accordance to the description in the Separation of AgNPs section, the AgNPs mass recovered from the solution was 36.4 mg (in 1 liter) in the sediment. By analyzing the Ag fractions in the original dissolution (Table 4), it was evidenced that it contained only 39.2% of the Total AgNPs (92.90 ± 4.48 mg/L as measured by the atomic absorption technique.

Antibacterial properties

To determine the minimum bactericidal concentration (MIB), AgNP dissolutions were prepared, from the sediment, in the range of 5, 10, 15 and 20 mg/L; Tryptic Soy Agar (TSA), was added after 20 minutes contact time of E. coli ATCC 25992 with the AgNPs solution. Fig. 7 shows the antimicrobial activity for the surviving CFUs for the dissolution prepared from the powder extracted.

For E. coli ATCC 25992, concentrations of 5 and 10 mg/L inhibited at least 99.88% of the initial CFU/mL hindering in fact all growth. The same trend appeared in the typical growth kinetics for concentrations in the range of 710-850 CFU/mL in which E. coli died at 15, 50 and 100 mg/L (Fig. 8). Plant extract wich containing only biomass of EC and 0 mg/L of AgNP not showed inhibitory activity.

Silver nanoparticles in the membrane of the bacteria as well as in its interior were observed

Table 3. Characteristics of AgNPs formed by different synthesis conditions.

	Average size (nm)	% AgNPs < 10 nm	Phase	Shape and Structure
pH 12	4.18 ± 3.9	92%	Ag AgO	Cubic, MTP, polyhedral, icosahedral, spherical, defective
pH 10	5.7 ± 2.6	93 %	AgO	
pH 7	6.9 ± 7.7	82%	Ag AgO	
pH 4	7.8 ± 9.6 nm	88.8%	AgO	

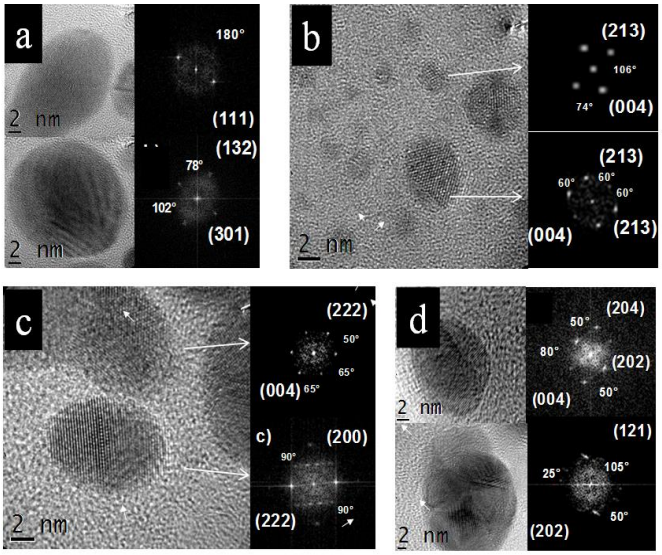

Fig. 6. Bright Field HRTEM images of AgNPs produced for the different pH conditions for leaves, (a) pH = 12, (b) pH = 10, (c) pH= 7 and (d) pH = 4.

by electron microscopy by Raffi et al., 2008 [30] and Lara et al., 2015 [31]. Consistent of this, in Fig. 8, shows the number of CFU reduced significantly with increasing the concentration of silver nanoparticles. The bacterial growth inhibition trend observed from CFU data has matched well with the results of optical density obtained.

The sensitivity to the AgNPs by the bacteria E. coli ATCC 25992 has been attributed to the structural differences in their external cell wall for the presence of lipopolysaccharides and the proportion of peptidoglycans [32, 33]. Both factors influence directly the permeability of the cell wall according to the following mechanisms: a) In the Gram (-) bacteria the lipopolysaccharides are the main component of the exterior membrane,

Table 4. Amount of Ag in mg/L measured by the atomic absorption technique, for the different fractions (Ag total, Ag in the supernatant and Ag NPs) for biosynthesis with Eichhornia crassipes leaves at pH12

Dissolution	Concentration mg/L	% in the dissolution
Ag-total	109.82 ± 3.59	100.0%
Ag in supernatant	16.92 ± 1.41	15.4%
AgNP	92.90 ± 4.48	84.6%

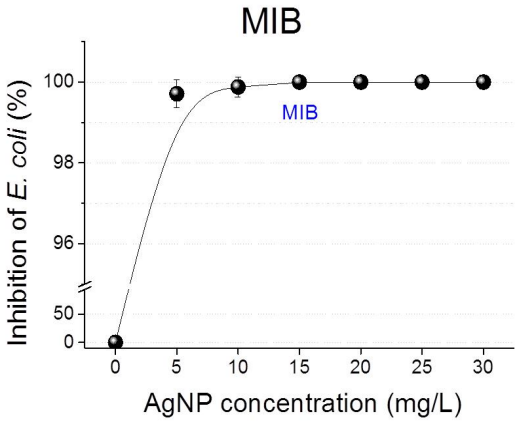

Fig. 7. CFU/mL E. coli ATCC 25992 and concentrations of AgNPs in MIB

nevertheless the presence of lipids -due to the hydrophobic nature- acts as a barrier towards many antibiotics and defense factors of the wall plant [32]. b) The AgNPs accumulate in the peptidoglycan fraction -a specific component in bacteria's cell wall- where the ion release is more efficient between underlying layers. The width of this component in the Gram (-) bacteria varies from 2-3 nm; this phenomenon produces a slower ionic release in bacteria such as E. coli [34 - 36].

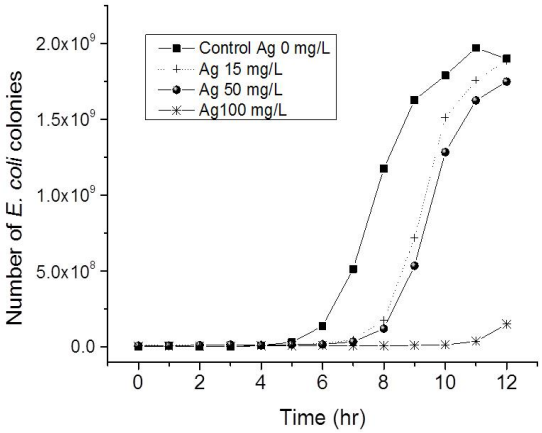

Fig. 8. Growth curves in TSA agar inoculated with of E.Coli ATCC 25992 bacteria in the presence of different concentrations of AgNPs <10nm synthesized.

Comparison between Ag antimicrobian dissolutions

Considering the previously determined MIB, the efficiency of the AgNPs (preparated from sediment) were compared with three Ag solutions: a) Commercial solution, b) $AgNO_3$ (both ionic Ag) and d) initial as prepared suspension obtained by bioreduction (Fig. 9).

In spite of the initial count for a given strain, the silver species (Ag^+ or AgNPs) contained in the antimicrobian agents influenced directly the bactericidal tests. The ionic Ag based agents were 100% efficient at 10 mg/L for E. coli, thus confirming the influence of Ag^+ ions (free and contained in the AgNPs) on the antimicrobial activity process [36 - 40].

The best test results were obtained on using the as prepared bioreduction suspension; where the AgNPs were in a proportion of 84.6%. The required dose was the lowest (5 mg/L for E. coli) as compared with the other tested agents that exceeds the effect produced by each silver species

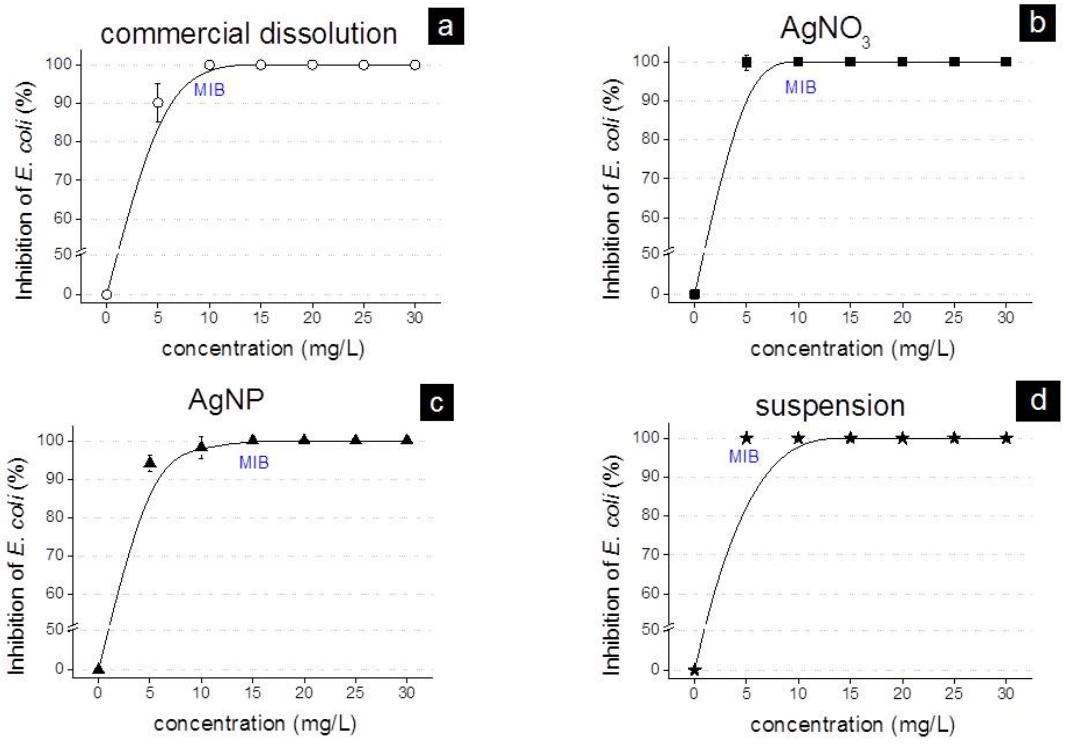

Fig. 9. Assays of inhibition (MIB) for different Ag solutions a) Commercial solution, b) $AgNO_3$, c) Solution obtained from the sediment only d) Initial as prepared suspension, added to E. Coli ATCC 25992. All experiments were performed in duplicate on three separate occasions. Results represent the means ± standard deviations (SD).

separately, while the surface phenomena present in the NPs due to their polyhedral morphology contribute to the efficiency of the germicidal agent.

One major disadvantage of using Ag^+ is its high reactivity towards chlorine, sodium, phosphates and organic materials. This is due that upon reaction with these elements, the Silver can become inactive due to complex formation or precipitation, amongst other phenomena [41, 42], therefore, the interest has fallen upon materials that don't react readily with the mentioned substances, such as AgNPs. Other studies Morones et al. (2005) [40] and Rai et al. (2009) [43], Pal et al., (2007) [44] reported similar results to the ones presented here using different synthesis techniques.

However in this study we have clearly established that the use of AgNPs obtained by bioreduction with Eichhornia crassipes with sizes below 10 nm (97.8% of the particles for pH12 and leaves) that posess a icosahedral, rhombohedric and cubic shape and structure, constitute an excelent alternative to other bactericidal agents, at least in the case of E. coli., bacteria member of the fecal coliform group and is a more specific indicator of fecal pollution than other fecal coliforms [45]

Two key factors have led to the trend toward the use of E. coli as the preferred indicator for the detection of fecal contamination, not only in drinking water, but also in other matrices as well: first, the finding that some fecal coliforms were non fecal in origin, and second, the development of improved testing methods for E. coli.

The results obtained here constitute evidence that AgNps produced by bioreduction methods with Eichhornia crassipes constitute a clear alternative to other Ag bactericides.

CONCLUSIONS

In this work the biosynthesis of Silver nanoparticles has been demonstrated using water hyacinth Eichhornia crassipes from a lake in the state of Mexico, this is considered as the world's worst aquatic plant. The present investigation was planned to explore the potential of Eichhornia crassipes in a way that, its positive attributes outweigh the negative ones.

Qualitative analysis of the plant parts have revealed the presence of various components of importance including tannins, phenolic and polyphenolic contents. The result obtained indicates that though the plant is an aquatic weed can also be exploited in the manufacture of bactericidal solutions. The antimicrobial activity of the plant extracts and phytochemicals was evaluated with E. Coli microorganisms, no evident bactericidal effects were found of the use of the plant alone. However, when used for the bioreduction of Ag and its use for bactericidal effects on E. Coli it has been shown to be highly effective. This constitutes a facile and inexpensive method for the production of Silver nanoparticles.

The amount of HT in each section of the plant seems, according to our results, to strongly influence the synthesis process. The highest amount of HT seems to promote a better defined size and morphology distribution of the AgNPs and it was clearly found to occur in the leaves biomass.

From the work reported here we can conclude that: The basis for the applications of water hyacinth is the chemical selectivity and as it has been evaluated, the high acidity is favorable for the inorganic removal in polluted waters but also for the metallic reduction by the biomass.The size distribution of the particles are based on the pH, having the smallest and homogeneus ones with pH12. This produced particles of around 5 nm with a single-mode distribution and a dispersion of 4 nm.The structure of these particles are fcc-like and multiply twinned for the smallest, and the biggest have defects and deformed configurations although clearly polyhedral; since the binding energy is not reduced for polyhedron shapes as other metals, when the particle size increase.

The controlled size procedure demonstrated in this work, opens the perspective to apply the large amount of active biomass of the water hyacinth in the synthesis process presented here, high efficiency was reached and selective conditions to produce a high proportion of Ag quantum dots nanoparticles. Considering that 95% of the plant is water and just 5% corresponds to biomass, the efficiency of the use of biomass is of the order of 75% after treatment.

The general morphology was determined by TEM, HRTEM, showing that particles seem to have in general a roundish shape, which in detail were shown to be polyhedral in nature. Using leaves biomass seems to promote AgNP with sizes which were below 10nm and without coalescence, the predominant phase was cubic AgO.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests. Furthermore, the mentioned received funding in the Acknowledgment section did not lead to any conflicts of interest regarding the publication of this manuscript.

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