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Antimicrobial Effect of Silk and Catgut Suture Threads Coated with Biogenic Silver Nanoparticles

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Abstract

Two bionanocomposites based on suture threads, silk-silver nanoparticles (Ag NPs) and catgut-Ag NPs, were prepared through a green chemistry methodology using *Chenopodium ambrosioides* (Mexican Epazote) as reducing agent. UV-Vis spectrophotometry (UV-Vis), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM), were used for their characterization. UV-Vis confirmed the synthesis of silver nanoparticles. Micrographs showed polydisperse, mostly spherical, Ag NPs attached to both suture threads. The bionanocomposites antimicrobial properties were evaluated through cultures and inhibition zones tests. The *Chenopodium ambrosioides*-assisted synthesized bionanocomposites have proved antibacterial effect against *S. aureus* and *E. coli* in both sutures (silk and catgut) and could be potentially useful for oral or periodontal surgery. There was no significant difference statistically in inhibition of *Staphylococcus aureus versus Escherichia coli*.

Keywords: nanotechnology, silver nanoparticles, antimicrobial suture, oral microorganisms, *Staphylococcus aureus*, *Escherichia coli*

1. Introduction

In recent years, nanotechnology has become an issue of major importance because of its wide range of applications in different disciplines [1]. Rapid advances in medicine and biomaterials have become evident [2]. Nanomedicine supports the diagnosis, monitoring, prevention,

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and treatment of diseases [3, 4]. Silver nanoparticles have been widely used because they have proven to have significant antimicrobial activity [5–7]; therefore, they play a significant role in the field of biological systems, and in modern medicine [8–11].

It is known that properties of silver nanoparticles depend on their size and shape, consequently, their controllable synthesis represents a key challenge to achieve their more desirable characteristics [12]. The conventional approaches of nanoparticle synthesis use highly toxic chemicals which result in toxic side effects upon administration [13]. Hence, an alternative method is required to overcome these toxic effects. According to green chemistry, there is an increasing necessity for industries to become more sustainable through developing more environmentally friendly products [14, 15]. Green chemistry offers biological approaches incorporating the use of plant extracts for the synthesis of silver nanoparticles [16, 17]. *Chenopodium ambrosiodes* (Mexican Epazote) is native to the Mesoamerican region, belongs to the *Chenopodiaceae* family. It has been utilized in traditional cuisine and folk medicine [18]. Epazote is an aromatic herb, which has diverse pharmacological applications in the treatment of influenza, cold and respiratory ailments, [19], it has been widely used as vomiting and antihelmintic [20], also in gastrointestinal problems and worms [21], and healing of skin ulceration, and anti-inflammatory and antitumor properties. Therefore, accordingly to our previous experience, the biomolecules present in epazote may act as reducing reagents of silver ions and as passivation agents of the biogenic silver nanoparticles.

Sutures used in oral surgery should avoid or limit bacterial colonization to those parts exposed to oral fluids [22]; nevertheless, they offer adhesion in their surface to bacteria, increasing the susceptibility to postoperative infections [23, 24]. Suture knots are believed to be the principal site of bacterial colonization [25]. Silk and catgut have been used for the closure of wounds with acceptable results. However, a suture-based on natural fibers may increase the risk for the development of infectious processes [26]. Once suture material becomes colonized, local mechanisms to avoid infection become ineffective [27], in addition, some of the oral pathogens are antibiotic resistant [28–30]. Thus, efforts have been made to add some antiseptics to sutures, such as triclosan and chlorhexidine [31], a few cases have confirmed allergies to chemical substances, though [32–35]. Current trends suggest that the direct drug delivery from the suture to the surgical site can improve recovery and patient comfort [36].

The aim of this study was to evaluate the antimicrobial effect of two natural suture threads coated with biogenic silver nanoparticles, against two main representative microorganisms of the Gram-positive and Gram-negative groups: *Staphylococcus aureus* and *Escherichia coli*.

2. Experimental

2.1. Synthesis of Ag NPs

Chenopodium ambrosioides was acquired from the surrounding fields and washed and dried in the shade at room temperature for 24 hours. The leaves were mashed to a powder and mixed to obtain a homogeneous sample. *Chenopodium ambrosioides* powder was used as a green-reducing agent. About 1gram of each powder was immersed in 100 mL of distilled water, and underwent a boiling process. Afterwards, the solution was filtered through a filter paper. A 10 mM silver nitrate solution ($AgNO_3$, Sigma-Aldrich) was prepared. Both solutions were mixed in a 1: 2.5 ratio to generate Ag NPs.

2.2. Formation of bionanocomposites

To follow the nanoparticles formation, UV-Vis analysis was carried out every hour, during 6 hours after preparing each solution (*vide supra*). After this time, suture threads (Silk and Catgut USP 3-0, Atramat®) were totally immersed in the solution for 1 hour, then taken out and dried at room temperature.

2.3. Characterization of Ag NPs and bionanocomposites

2.3.1. UV-Vis spectroscopy

UV-Vis spectra measurements were recorded on a Cary 5000 UV-Vis-NIR Scanning Spectrophotometer using a quartz cell and the wavelength range from 300 to 600 nm.

2.3.2. SEM analysis

Assessment of Ag NPs impregnated suture threads was performed through scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) analysis in a JSM-6510-LV microscope (JEOL) at 20 kV of acceleration and using secondary electrons.

The samples were coated with a thin film of gold (c.a. 20 nm) using a Denton Vacuum DESK IV sputtering equipment.

2.3.3. TEM analysis

Shape and size of silver nanoparticles solution were evaluated with a Transmission Electron Microscope (TEM, JEOL JEM-2100-Tokyo, Japan). The specimens were sonicated during 4 hours to detach the nanoparticles from the fibers. Samples for the TEM observation were prepared by placing a drop of the sample solution on a copper grid (300 mesh) coated with carbon film and let it dry at room temperature. A 200 keV-accelerating voltage, was used in Brightfield mode, and high resolution.

2.4. Determination of the antibacterial activity of bionanocomposites

Streptococcus aureus and *Escherichia coli* strains were obtained from the Biochemistry Laboratory of the School of Dentistry, at the National Autonomous University of Mexico (UNAM). They were characterized by appropriate biochemical tests, and cultured by the agar well diffusion method, first, on a selective agar; mannitol salt agar or eosin methylene blue agar (EMB) respectively, and then in Muller Hinton agar plates.

To determine the antibacterial effect, paper discs were put on Petri plates. In each plate, three different paper disks were placed, embedded in the silver nanoparticles solution, a paper disc containing the infusion of *Chenopodium ambrosioides* was used as a control, and a bared paper disc was used as a blank control. Each plate was prepared in triplicate.

Silk and Catgut suture threads were cut into pieces of approximately 10 mm in length and put on the petri plates, prepared as previously described. Each plate contained: one suture thread (silk and catgut separately) embedded in the silver nanoparticles solution, and a silk suture thread (silk and catgut separately), without Ag NPs, used as blank control. Each plate was prepared in triplicate. The plates were incubated at 37°C in a Felisa® incubator for 24–48 hours. After incubation lapse, the diameter of the bacterial zone of inhibition was measured in millimeters.

3. Results

A characteristic and well-defined plasmon-band for silver nanoparticles was obtained at around 455 nm, as shown in **Figure 1**. Ag NPs synthesized by *Chenopodium ambrosioides* produced polydisperse and stable nanoparticles.

Transmission electron microscopy (TEM) study demonstrated the size and the spherical or quasi-spherical shape of biogenic Ag NPs (**Figure 2**). The average particle size was determined to be around 8 nm, as shown in **Figure 2**.

Scanning electron microscopy (SEM) micrographs revealed that Ag NPs were formed on the surface of both suture threads, silk and catgut (**Figure 3**). It is possible to assume that the cavities in the suture threads can act as nanoreactors to attach, firstly, the silver ions to subsequently form the nanostructures when the reducing agent acts.

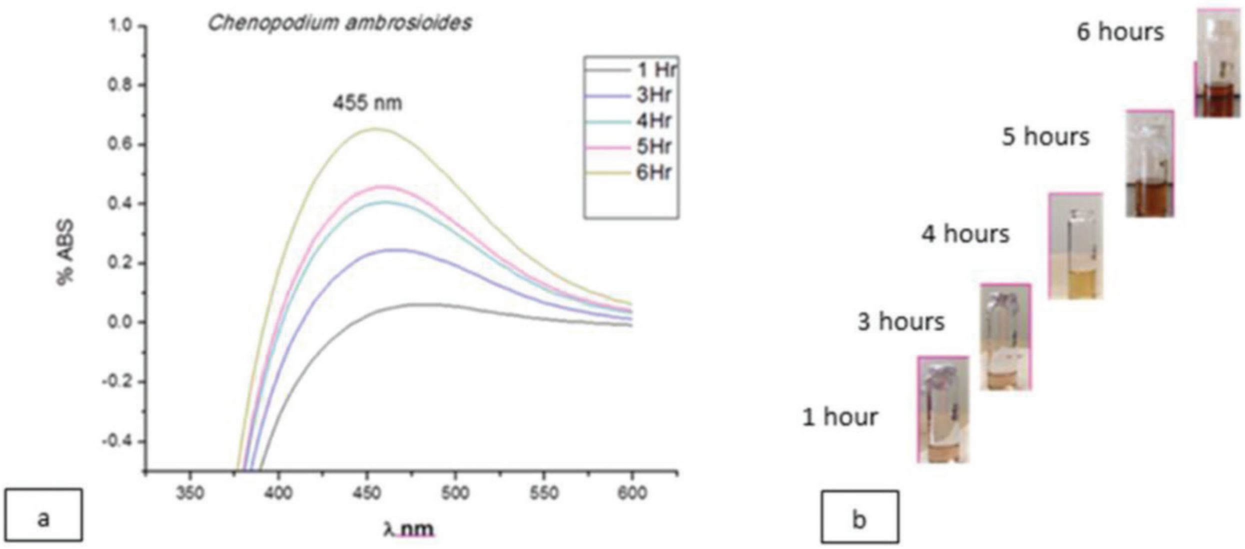

Figure 1. (a) UV-Vis Spectra showing that the surface plasmon resonance wavelength lies around 455 nm in Ag NPs synthesized by *Chenopodium ambrosioides*. (b) The flasks show the color change upon formation of silver nanoparticles; the solution changed from a light yellow to a light brown.

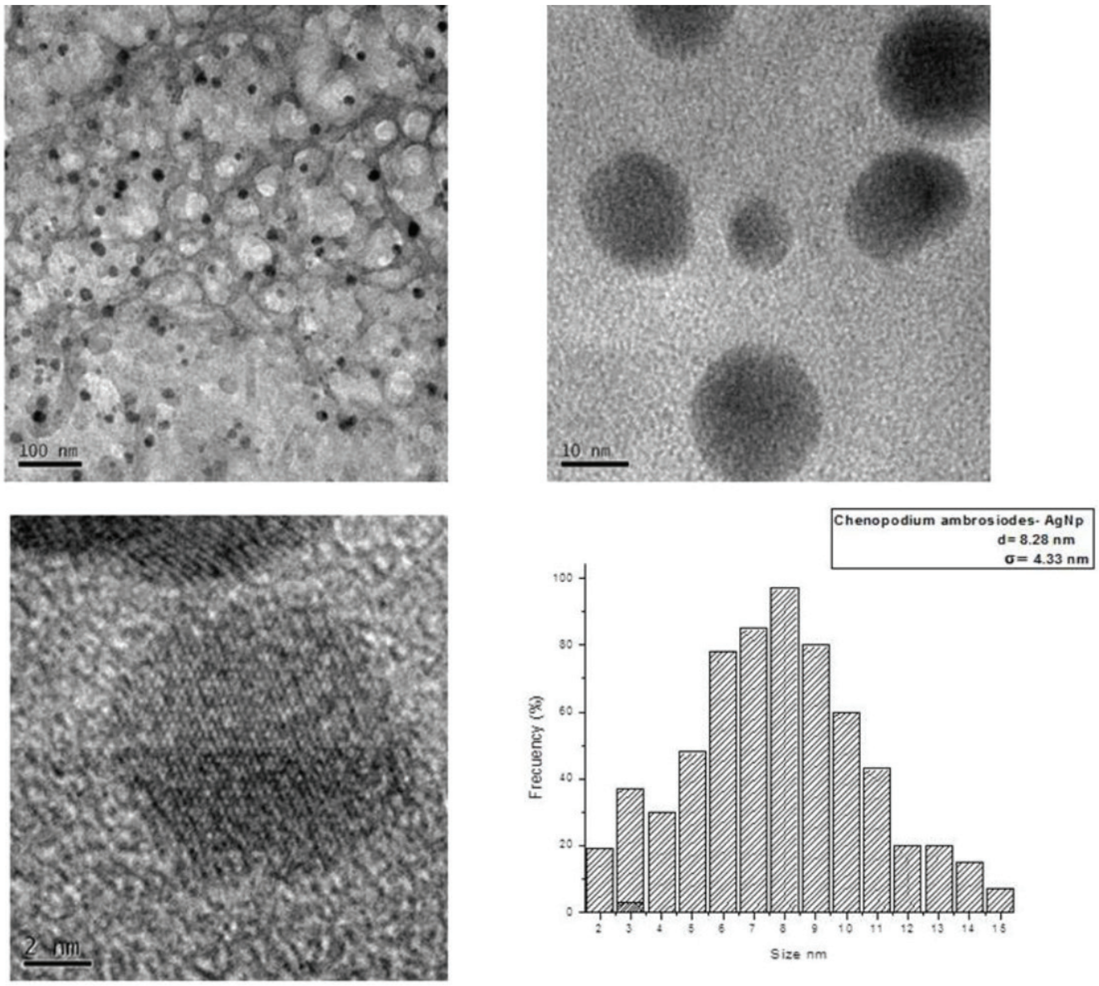

Figure 2. TEM images show that Ag NPs have a spherical shape, and the particle size distribution histogram shows that Ag NPs synthesized by *Chenopodium ambrosiodes* have a mean diameter of approximately 8 nm.

The antimicrobial activity of the biogenic Ag NPs, generated using *Chenoposium ambrosioides* infusion as reducing and capping agent, against *Staphylococcus aureus*, can be seen in **Figure 4**. The antibacterial effect of these biogenic Ag NPs can be observed clearly by the inhibition halo formed around the disk impregnated with the nanoparticles solution.

In **Figure 5**, the antimicrobial activity of both bionanocomposites against *S. aureus* and *E. coli* is observed. The suture threads (silk and catgut) were cut into small pieces and put on the Petri dishes, as mentioned in the experimental section. Some suture threads samples were used as a blank.

The inhibition growth of bacteria by the biogenic Ag NPs and the two bionanocomposites are shown in **Table 1**, revealing a strong antimicrobial effect of silver nanoparticles embedded in the corresponding suture threads against Gram-positive and Gram-negative bacteria.

As can be seen in **Table 1**, the growth inhibition of *Staphylococcus aureus* by disks impregnated with biogenic Ag NPs was on average 2.75 mm, compared to its control (*Chenopodium ambrosiodes* infusion with the same concentration). Also, the growth inhibition of *Escherichia coli* was

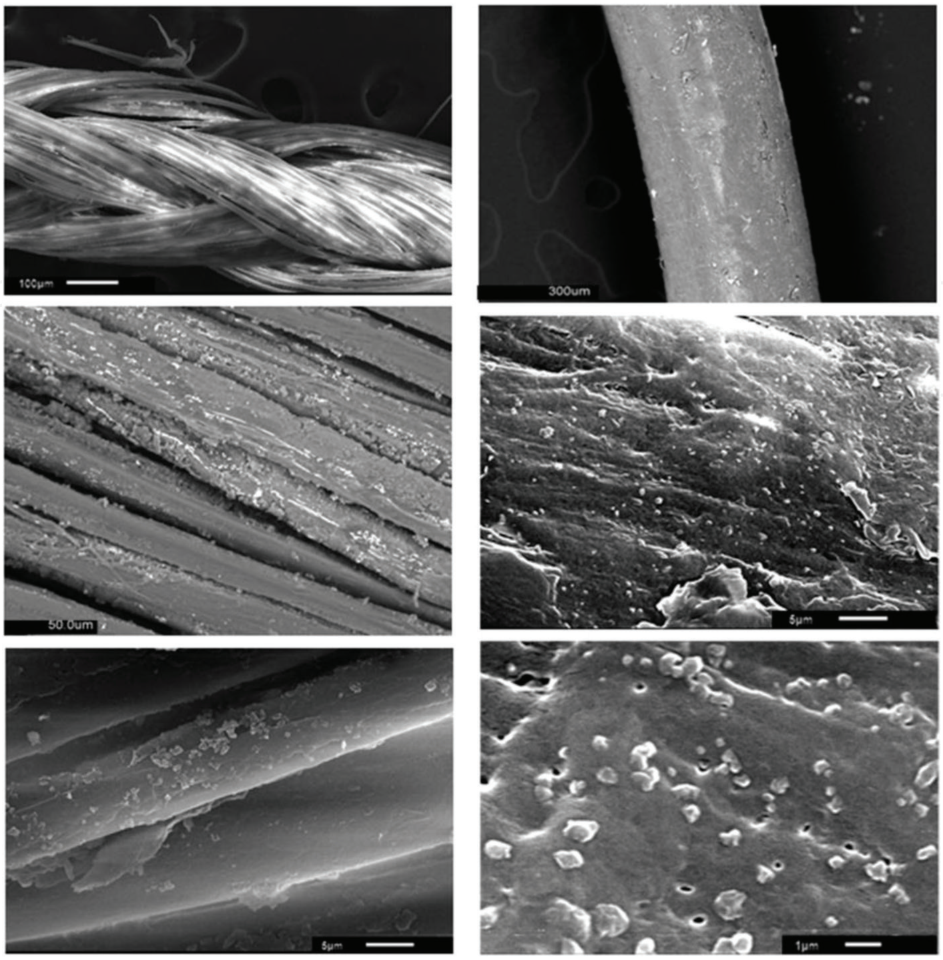

Figure 3. SEM micrographs showing Ag NPs embedded on silk (left images) and on catgut (right images) suture threads.

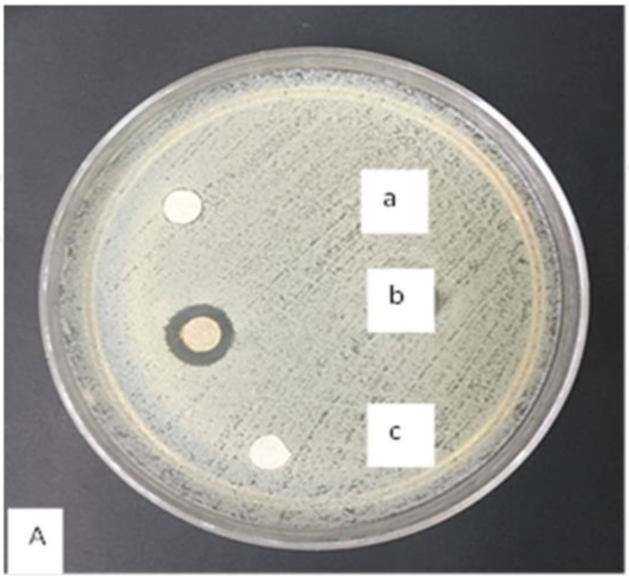

Figure 4. Ag NPs against *Staphylococcus aureus:* (a) blanc disc, (b) disc containing Ag NPs synthesized by *Chenopodium ambrosioides*, (c) disc with *Chenopodium ambrosioides* infusion as a control.

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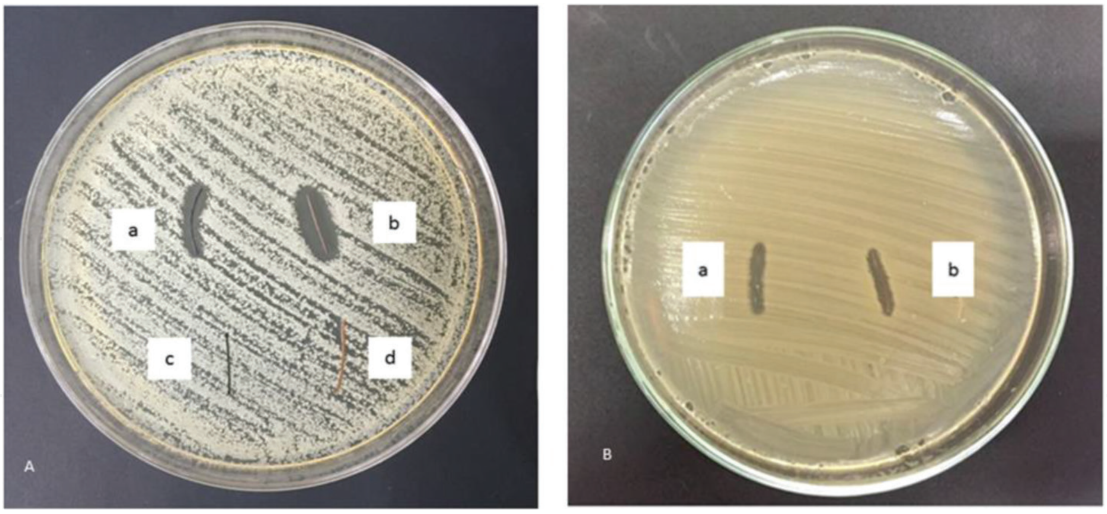

Figure 5. (A) Antibacterial effect of bionanocomposites against *Staphylococcus aureus*. (a) Silk suture thread-Ag NPs; (b) catgut suture thread-Ag NPs; (c) silk suture thread; (d) catgut suture thread. (B) Antibacterial effect of bionanocomposites against *Escherichia coli*. (a) Silk suture thread-Ag NPs; (b) catgut suture thread-Ag NPs.

Table 1. Inhibition of bacterial growth

	Staphylococcus aureus					Escherichia coli				
Discs and sutures	n	Mean (SD)		Minimun Value	Maximum Value	n	Mean	SD	Minimun value	Maximum value
AgNP C.a.	4	2.75 (0.5)%		2	3	4	2.5	0.577	2	3
Control C.a.	4	0	0	0	0	4	0	0	0	0
Blank control	4	0	0	0	0	4	0	0	0	0
Silk AgNP C.a.	15	2.533	0.516	2	3	15	2.467	0.516	2	3
Catgut AgNP C.a.	15	2.667	0.488	2	3	15	2.467	0.516	2	3

Table 1. Inhibition of bacterial growth.

on average 2.5 mm, compared to its control (*Chenopodium ambrosiodes* infusion with the same concentration). With the use of silk impregnated with biogenic Ag NPs the growth inhibition of *Staphylococcus aureus* was on average 2.53 mm, compared to the control solution. When silk impregnated with biogenic Ag NPs was used, the growth inhibition of *Escherichia coli* was on average 2.46 mm, compared to its control solution. For catgut impregnated with biogenic Ag NPs, the growth inhibition of *Staphylococcus aureus* was on average 2.6 mm; and the growth inhibition of *Escherichia coli* was on average 2.46 mm. There was no growth inhibition with blank or control discs, neither with silk and catgut blank sutures, as can be seen in **Figures 4** and **5**. All the measurements were replicated three times for each treatment. Minimum and maximum value for each Petri sample is shown. The effect on both types of bacteria was similar. No statistically significant differences were found in both sutures with Ag NPs embedded.

4. Discussion

Periodontal and oral surgical procedures, in combination with the presence of foreign materials, may develop infectious processes when bacteria lodge on the suture material invading the suture track [22]. In the oral cavity, sutures are placed within high vascularity tissues, in a moist bacteria-rich environment. When performing surgical procedures using natural sutures, such as catgut or multifilament threads, as silk, the risk of infection may increase because bacteria are housed in the interstices [23]. Therefore, the choice of the kind of material for the closure of surgical wounds is paramount. Catgut, a monofilament suture made from the submucosa layer from the intestines of animals has been banned in Europe and Japan, due to health concerns [24, 25]. Silk is a natural protein fiber created by the *Bombyx mori* silk worm. It is well known for its water absorbency, which may favor bacterial growth [26]. Although both types of sutures have suitable properties for use, such as biocompatibility, flexibility and endurance, and sutures are designed to meet different needs, nowadays, more surgeons are opting for the use of synthetic suture materials [27]. We believe that the use of a suture thread bionanocomposite could prevent the colonization of pathogenic microorganisms. In this context, Ag NPs were selected in this study for decorating conventional sutures. It has been proved that the use of chemical elements such as silver is an alternative to multiple microorganisms. Silver is a nontoxic, safe inorganic antibacterial agent that can kill about 650 types of disease-causing microorganisms; silver nanoparticles can inhibit bacterial growth [5, 28]; therefore, they are currently being used in a variety of potential applications in pharmaceutics, medicine [6, 29] and we have recognized its use in dentistry [30, 31].

Although the precise antibacterial action of silver nanoparticles is not completely understood, it is believed that electrostatic attraction between negatively charged bacterial cells and positively charged nanoparticles is important for their antibacterial activity [32]. Ag NPs can interact with disulfide bonds of the glycoprotein/protein contents of microorganisms such as viruses, and bacteria, exerting an antimicrobial effect on Gram-positive and Gram-negative producing lysis in the peptides of the membrane of microorganisms. Certain studies have proposed that Ag NPs prompt neutralization of the surface electric charge of the bacterial membrane and change its penetrability, leading to bacterial death [33, 34]. On the other hand, the size of bacteria is measured in microns, three orders of magnitude greater than the nanoparticles obtained by green synthetic methods; therefore, the probability that the nanoparticles meet bacteria is higher when the size of Ag NPs is smaller. As it is known, the properties of Ag NPs depend on their size and shape, consequently, their controllable synthesis represents a key challenge to achieve their more desirable characteristics [13]. The development of reliable, eco-friendly processes for the synthesis of nanomaterials is an important aspect of nanotechnology [35]. The synthesis of Ag NPs by eco-friendly agents represents an environmental and economically sustainable biological method that minimizes the costs and provides the benefits and properties of native plants and herbs such as *Chenopodium ambrosioides.* We have studied that there is huge potential of Mexican medicinal plants [36], among them, *Chenopodium ambrosioides* (Mexican Epazote) has been utilized in traditional cuisine and folk medicine since ancient times [18], it has demonstrated diverse pharmacological applications, it is also useful for healing of skin ulceration, and shows anti-inflammatory and antitumor properties [19, 37]. It is difficult to assign a single component of being responsible for the bioreduction; however, it is considered that the main chemical constituents involved in this process are mainly monoterpenoids, sesquiterpenoids, and flavonoids, among others [38].

To understand the way by which the nanoparticles adhere to the suture threads, it is necessary to describe the composition of the threads. Catgut was the first bioabsorbable suture made from animal intestines braided together to constitute a single strand, consisting mainly of collagen [39]. The collagen helix is a type of secondary structure protein consisting of amino groups. It is well known that silk is formed by two main proteins (sericin and fibroin). Fibroin consists mainly of recurrent amino acids sequence, containing carboxylic functional groups besides amino groups. Both show affinity to metallic atoms and cations, also, possess reducing properties. For this reason, carboxylic and amino groups could be responsible for the stabilization and capping of the silver nanoparticles [40].

It is important to consider that by electrostatic attraction the silver nanoparticles remain adhered to the threads, ensuring that there are not released into the oral environment. Besides, high surface area to volume ratio, allows Ag NPs to be effective in very small amounts [41], thus, we consider that the use of these type of bionanocomposites is an alternative approach to combat the bacterial resistance toward conventional antibiotics [42, 43], reducing as much as possible, the exaggerated prescription antibiotic schemes, affecting the systemic health of the patient; nonetheless, *in vivo* studies are required.

5. Conclusions

One of the pillars of oral surgery is based on a surgical procedure in an aseptic field, so it is essential to implement all means to achieve it, the bionanocomposites here presented can be effective for the treatment of periodontal surgery, being a useful tool against resistant bacteria. In this study, *Chenopodium ambrosiodes* turns out to be an appropriate reducing agent for coating natural suture threads with Ag NPs. The formed bionanocomposites possess important antibacterial activity against *S. aureus* and *E. coli.* This is an option that may help to reduce the harmful effect of the major pathogens while representing an attractive option as part of the eco-friendly materials, which would result not only in less expensive drugs but also in substances with a minor risk to human health and the environment.

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Conflict of interest

The authors declare that they have no conflict of interest.

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