Chapter

Green Synthesis of Silver Nanoparticles Using *Heterotheca inuloides* and Its Antimicrobial Activity in Catgut Suture Threads

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Abstract

Silver nanoparticles were synthesized through a green method, using *Heterotheca inuloides* as a bioreducing agent. Moreover, catgut suture threads were decorated with those biogenic silver nanoparticles, and their antibacterial activity versus highly resistant pathogenic microorganisms was evaluated. The principles of green chemistry and nanotechnology allow us to obtain advanced materials, such as suture threads, which can reduce or avoid the prevalence of infectious processes in the medical field. Mexican medicinal plants, such as *H. inuloides*, represent an adequate alternative for biosynthesis; this plant species is known for its medicinal benefits and its antibacterial activity, and for that reason, it is being used in folk medicine.

Keywords: *Heterotheca inuloides*, green synthesis, silver nanoparticles, antimicrobial activity, catgut, suture

1. Introduction

Diverse green synthesis methods, involving the use of plant extracts as reducing agents, provide attractive approaches to synthesize AgNPs.

Mexican medicinal plants represent an adequate alternative for biosynthesis, such is the case of *Heterotheca inuloides*, a plant known for its medicinal benefits, as well as anti-inflammatory and analgesic properties. The plant, commonly named as Mexican arnica has been traditionally used for its antimicrobial activity, antifungal, cytotoxic and antioxidative properties, leading the World Health Organization (WHO) to recognize its use in medicine. This species has also been used to treat dental diseases and gastrointestinal disorders [1–6].

The wide use of *H. inuloides*, in medicine, can be attributed to its more than 140 components. Several constituents of the aqueous extract obtained from the dried flowers have been identified and characterized as antibacterial agents. Flavonoids, sesquiterpenoids, triterpenoids, and sterols are mainly present on its chemical composition [7–9].

Conventional approaches in nano-synthesis involve the use of highly toxic chemicals, resulting in side effects after administration [10, 11]. For this reason, it is of utmost importance for biomedical science to try to minimize any consequent risk to human health.

In modern surgery, attempts have been made to reduce the prevalence of infections related with surgical sutures, through the use of coated materials [12]. Nevertheless, the risk of surgical site infection is a constant challenge in wound closure. By using sutures with an antibacterial coating, the risk of infection is considerably reduced. The significant feature of silver is its broad antimicrobial spectrum associated with biomaterial-related infections [11, 13].

We present a total green synthetic method where *Heterotheca inuloides* is used for the first time to decorate catgut, a suture thread widely used in surgery. Its characterization, and antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, is reported.

2. Experimental

2.1 Synthesis of AgNPs

The plant material was collected from surrounding fields of the State of Mexico, and it was cleaned using tap water followed by distilled water. *H. inuloides* leaves were dried and finely ground to a powder and then kept at room temperature for 24 h. About 1 gram of powder was poured in 100 mL of distilled water and boiled. The solution was filtered using filter paper. A 10 mM silver nitrate solution ($AgNO_3$, Sigma-Aldrich) was prepared to generate AgNPs; both solutions were mixed in a 1:2.5 ratio.

After 6 h, catgut (USP 3-0, Atramat®) was totally immersed in the solution for 1 h and then taken out and dried at room temperature.

2.2 Characterization of AgNPs

2.2.1 Spectrophotometry by UV: Vis

UV-Vis was performed every hour for the next 6 h. Spectral measurements were recorded on a Cary 5000 UV-Vis-NIR Scanning Spectrophotometer using a quartz cell, and the wavelength ranges from 300 to 600 nm.

2.2.2 Spectrophotometry by FTIR

The FTIR analysis was performed (Bruker, Model 27) to identify the main functional groups in the aqueous extract of *Heterotheca inuloides*.

2.2.3 Scanning electron microscopy (SEM)

Catgut samples were prepared for its analysis in a JSM-6510-LV microscope (JEOL Tokyo, Japan) 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.

2.2.4 Transmission electron microscopy (TEM)

Transmission electron microscope (TEM, JEOL JEM-2100, Tokyo, Japan) was used. To evaluate shape and size of silver nanoparticles, the solution was analyzed by placing a drop on a copper grid (300 mesh) coated with carbon film and let to dry at room temperature. A 200 kiloelectronvolt accelerating voltage was used in bright-field mode and high resolution.

2.3 Antibacterial activity

The antibacterial activity of AgNPs was determined by well diffusion method against the *Staphylococcus aureus* and *Escherichia coli* on the Mueller-Hinton agar plates.

Catgut suture threads were cut into pieces of approximately 10 mm of length and put on the Petri dishes. Each plate was prepared in triplicate. The plates were incubated at 37°C in a Felisa® incubator for 24–48 h.

After incubation, a clear zone appeared, and by measuring the halo of inhibition for both strains, the antibacterial effect was assessed.

3. Results

3.1 UV-Vis spectroscopy

AgNPs synthesized by *Heterotheca inuloides* produced polydisperse and stable nanoparticles as shown in **Figure 1**. The increase in the intensity of surface plasmonic resonance, at 451 nm, as a function of time, is observed. In addition, it is confirmed that at 6 h, the formation of the nanoparticles is finished.

By means of transmission electron microscopy (TEM), the size and shape of AgNPs are demonstrated; an average nanoparticle size of 16.0 nm and a standard

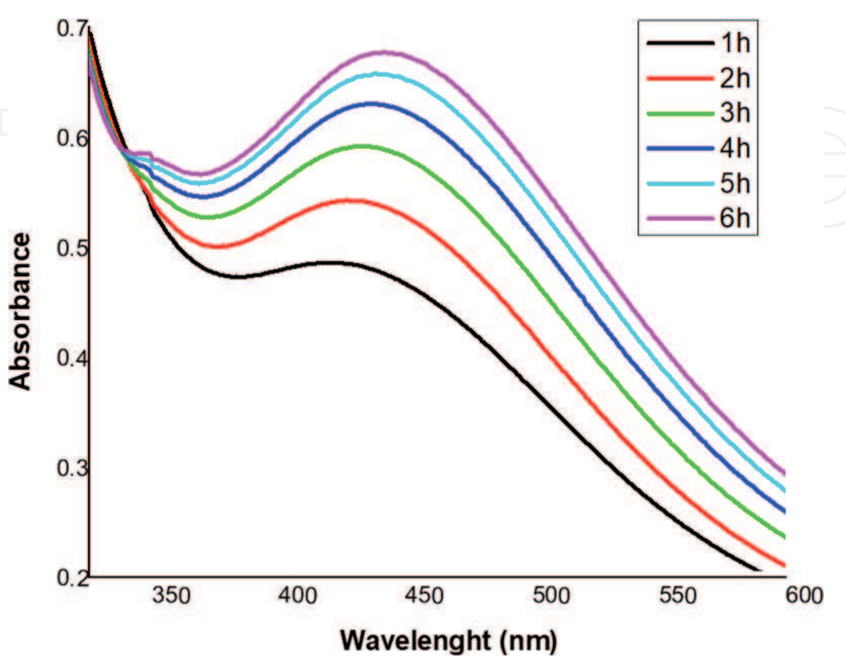

Figure 1.
UV-Vis spectra showing that the AgNP plasmon wavelength lies between 440 and 460 nm.

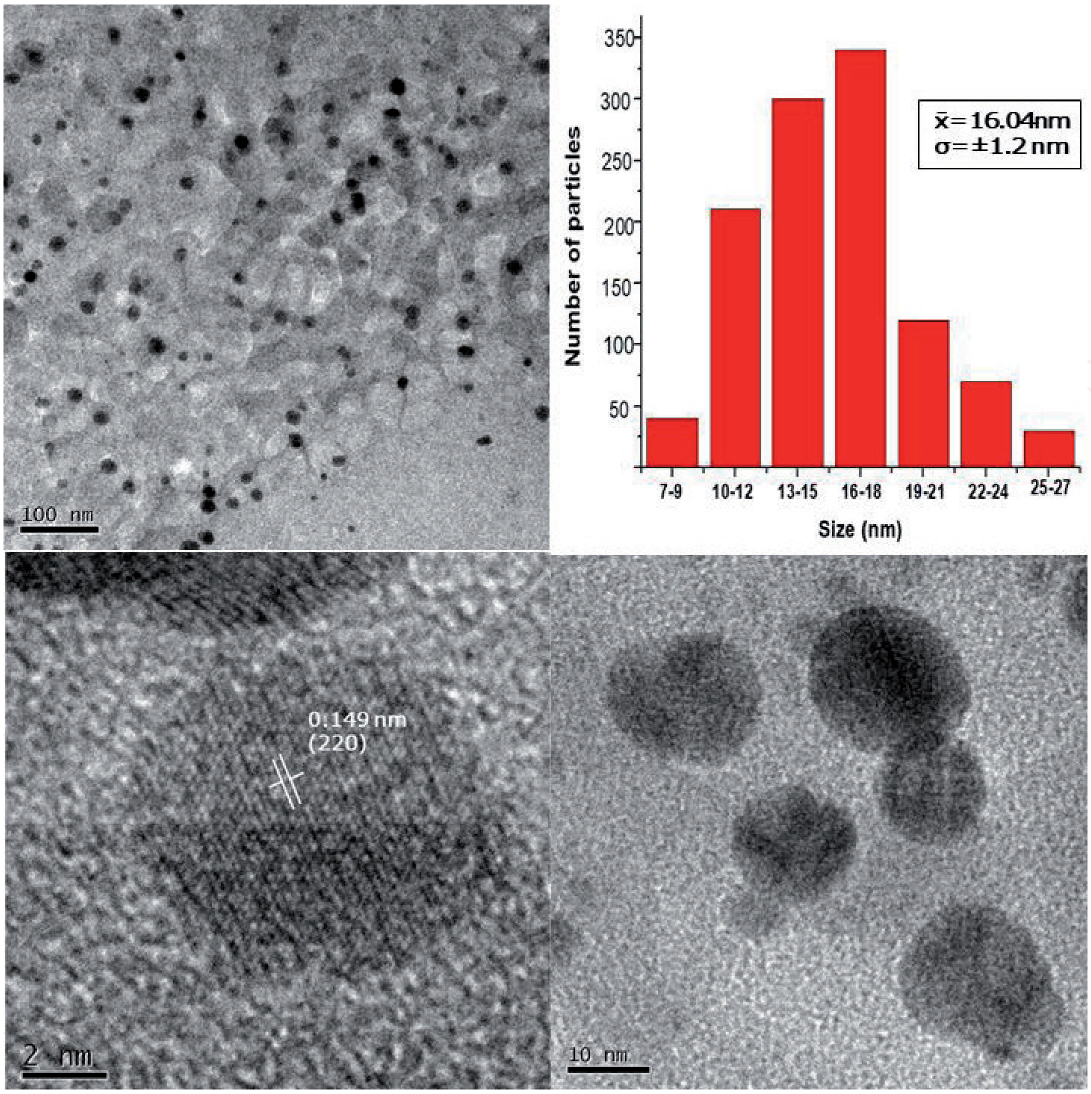

Figure 2.
TEM images show the size distribution and a spherical shape of AgNPs synthesized by the green reducing agent, having a mean diameter of approximately 17 nm.

deviation of 1.2 nm are recognized; in addition, an interplanar distance of 0.149 nm, corresponding to plane (220 nm), was found (**Figure 2**).

Scanning electron microscopy images of catgut embedded with AgNPs (reduced with *Heterotheca inuloides*) at different magnifications are shown in **Figure 3**. Ag nanoparticles of spherical in shape are observed distributed over the fiber surface.

4. Characterization of bioreducing agent of AgNp by infrared spectroscopy

H. inuloides represents a source of chemical compounds with variable structural patterns. Several different types of compounds such as sesquiterpenes, triterpenes, polyphenols, and phytosterols have been isolated from essential oil and organic extracts from various parts, including roots, aerial parts, and flowers. According to the abovementioned, the following characteristic functional groups, 3268 cm^{-1} (–OH), 2942 cm^{-1} (c (sp2)–H), 1584 cm^{-1} (C = O), 1393 cm^{-1} ($-CH_2$), 1258 cm^{-1} ($-CH_3$), 1033 cm^{-1} (CO), and 595 cm^{-1} (CH), were detected (**Figure 4**).

The antimicrobial activity of the infusion using *Heterotheca inuloides* as reducing agent, against *Staphylococcus aureus*, can be seen in **Figure 5**. A well-defined inhibition halo around the disk impregnated with the nanoparticles solution is visible.

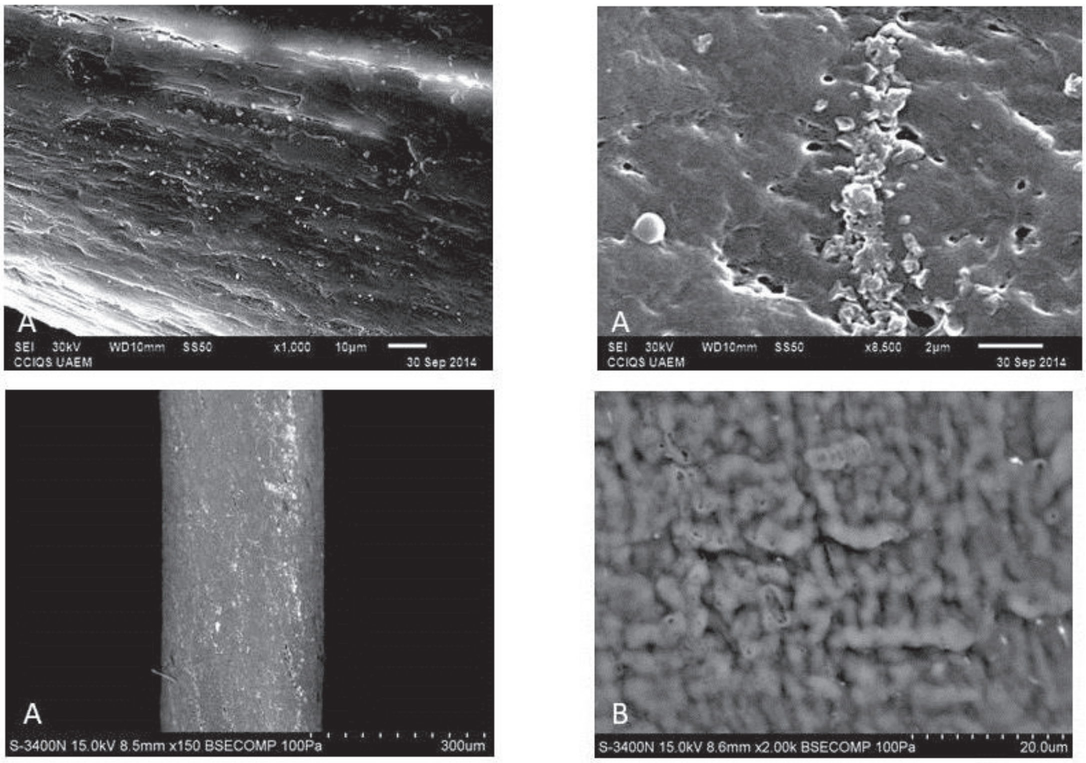

Figure 3.
SEM micrographs showing the catgut suture threads coated with AgNPs synthesized by the green reducing agent. (A) Images revealed that AgNPs were formed on the surface. (B) The micrograph shows a plain catgut suture.

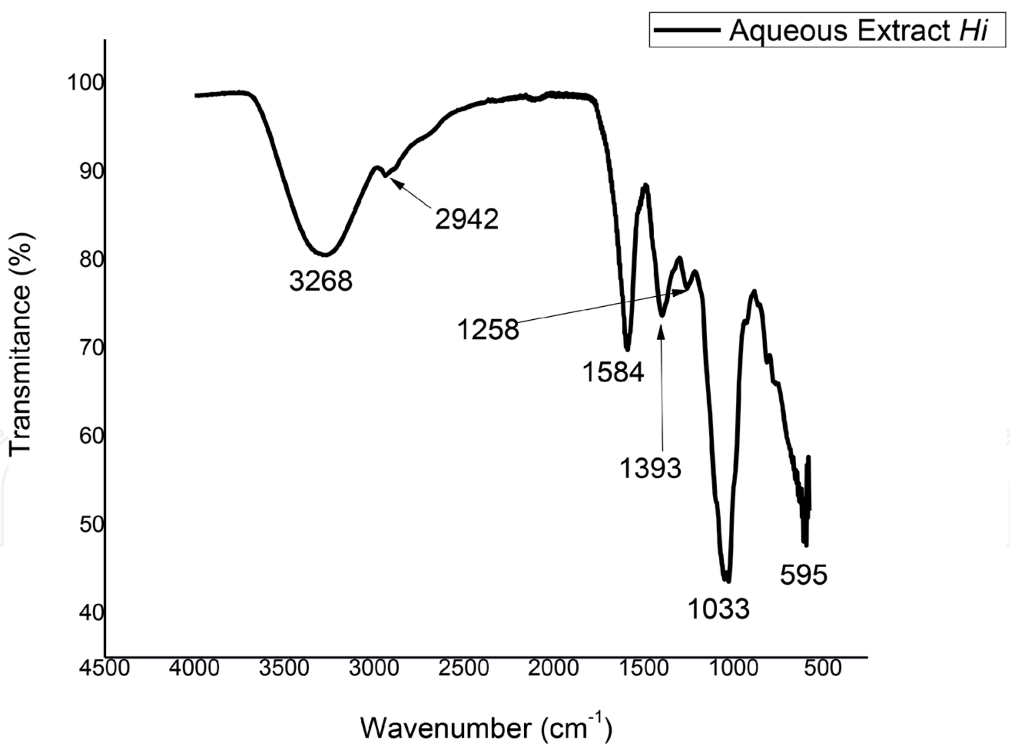

Figure 4.
FTIR spectrum of the Heterotheca inuloides *aqueous extract.*

In **Figure 6**, the antimicrobial activity of catgut against *S. aureus* and *E. coli* is observed. The suture threads were cut into small pieces and put on the Petri dishes. Some suture thread samples were used as blank.

The inhibition zone for both strains is presented in the next tables.

Table 1 shows that the use of *Heterotheca inuloides* to synthesize AgNPs produces an antibacterial effect against both strains, by testing disks. The growth inhibition

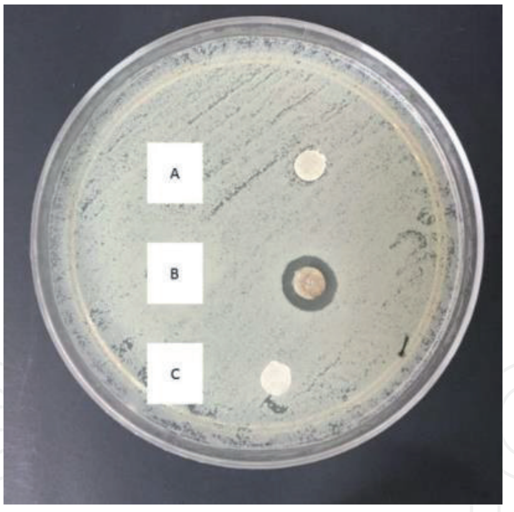

Figure 5.
AgNPs against Staphylococcus aureus: *(A) blank disk, (B) disk containing AgNPs synthesized by* H. inuloides, *and (C) disk with* H. inuloides *infusion as a control.*

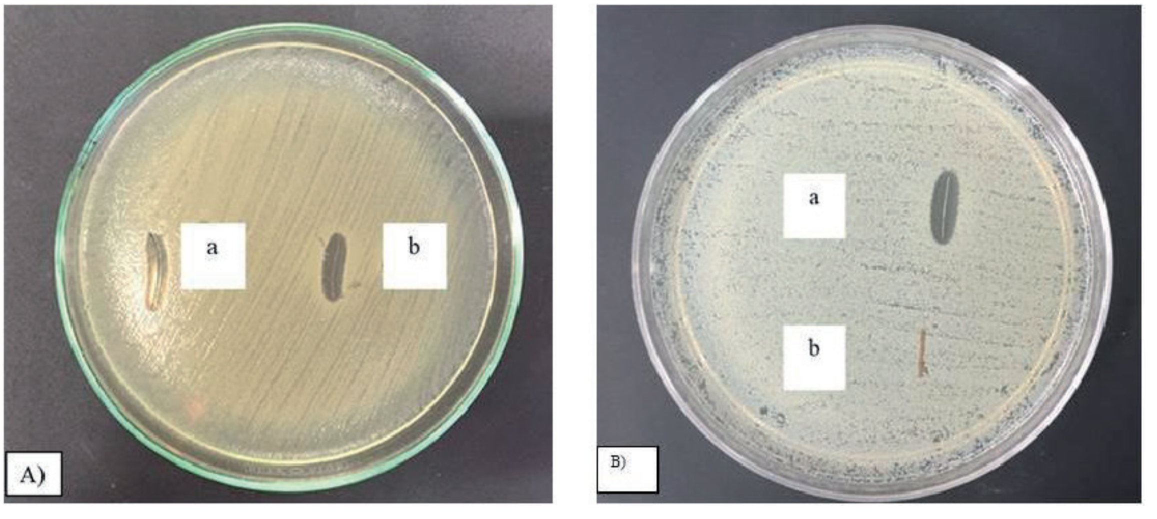

Figure 6.
Antibacterial effect of suture against both strains a and b. (A) Inhibitory halo of catgut suture with AgNPs versus E. coli. (B) Inhibitory halo of catgut suture with AgNPs versus S. aureus. (a) Catgut with AgNPs. (b) Catgut without AgNPs used as a blank.

halo for *S. aureus* was on 3.5 mm average. While for *E. coli*, it was 3.25 mm on average. Without having a statistically significant difference between both strains.

When performing the suture inhibition test for both strains, an average inhibition zone of 3.46 mm for *S. aureus* and an inhibition zone of 2.8 mm for *E. coli* can be seen in **Table 2**, demonstrating a statistically significant difference and a greater zone of inhibition with the use of catgut versus *S. aureus*.

There was no growth inhibition with blank or control disks, neither with catgut blank sutures. All the measurements were replicated three times for each treatment.

5. Discussion

Regarding the UV-Vis results, we can recognize the presence of the characteristic surface plasmonic resonance of silver nanoparticles as other authors have reported to appear from 400 to 500 nm [14].

Other authors have shown that silver nanoparticles with sizes smaller than 50 nm offer high antimicrobial activity [15] that supported on catgut fibers and obtain a

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Variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]
Sdis~pHi	4	3.5	0.2886751	0.5773503	2.581307–4.418693
Edis~pHi	4	3.25	0.25	0.5	2.454388–4.045612
combined	8	3.375	0.1829813	0.5175492	2.942318–3.807682
diff		0.25	0.3818813		-0.6844299–1.18443
diff = mean(Sdisco_NpHi) − mean(Edisco_NpHi)				t = 0.6547	
Ho: diff = 0				degrees of freedom = 6	
Ha: diff < 0		Ha: diff != 0		Ha: diff > 0	
Pr(T < t) = 0.7315		Pr(|T| > |t|) = 0.5370		Pr(T > t) = 0.2685	

Table 1.
Measures of the zones of inhibition of the disks against S. aureus *and* E. coli.

Two-sample t test with equal variances					
Variable	**Obs**	**Mean**	**Std. Err.**	**Std. Dev.**	**[95% Conf. Interval]**
Scatgu~i	15	3.466667	0.1652319	0.6399405	3.112279–3.821054
Ecatgu~i	15	2.8	0.2225395	0.8618916	2.3227–3.2773
combined	30	3.133333	0.1495844	0.8193072	2.827399–3.439268
diff		0.6666667	0.2771739		0.0989016–1.234432
diff = mean(Scatgut_NpHi) − mean(Ecatgut_NpHi)				t = 2.4052	
Ho: diff = 0				degrees of freedom = 28	
Ha: diff < 0		Ha: diff != 0		Ha: diff > 0	
Pr(T < t) = 0.9885		Pr($\lvert T\rvert > \lvert t\rvert$) = 0.0230		Pr(T > t) = 0.0115	

Table 2.
When comparing both strains; there was a mean inhibition zone of 3.4 mm against S. aureus. *While for* E. coli *there was a mean inhibition zone of 2.8 mm representing a statistical significant difference of 0.0230 value.*

double function, in **Figure 3**. The accommodation of the nanoparticles on the surface of the fibers can be observed, and some authors have observed this same behavior [16].

Also, the main functional groups present in the reducing agent are recognized [1], which makes it possible to obtain silver nanoparticles with average sizes of 16.04 nm.

According to the Centers for Disease Control and Prevention, assessment of wound healing after a surgical procedure is important [17]. Infection is the most important and preventable cause of impaired wound healing. Microorganisms can rapidly reach tissues after surgery [18].

A surgical site infection (SSI) surveillance in oral or maxillofacial surgery is necessary because the mouth is widely colonized by highly pathogenic microorganisms. Besides, suture threads are placed in a moist environment [19]. One of the categories to classify the SSI is the complications related to the superficial incision, in which the suture material used may be related [20]. Whether its natural or synthetic origin, the sutures are strange to the body and therefore cause tissue reaction. Any suture may provide an environment conducive to the propagation of infection [21].

In 2002 the Food and Drug Administration (FDA) approved the use of the first suture coated with antimicrobial and triclosan (polyglactin 910-polychlorophenoxyphenol), which has a broad-spectrum activity against Gram-positive and Gram-negative microorganisms [12].

However, the resistance of highly pathogenic microorganisms has been reported as a disadvantage of the use of triclosan [22].

An advanced material for the closure of a wound, with direct drug delivery from the suture to the surgical site, may be the key for the prevention of infections.

Mexico is one of the five megadiverse countries of the world [23]. Pharmaceutical products derived from this plant are widely used, since ancient times, due to the botanical exploration of the Valley of Mexico, one of the most well-known regions from the scientific and botanical aspect [24]. The Ministry of Health has studied its traditional therapeutic use in most regions of the country, using the flower as an infusion and other presentations, [25] searching for the establishment of public policies, and recognizing the importance of epidemiological contributions of popular medicine [26].

Plants have different types of metabolites that can help in the reduction of silver ions. The biological methods using plant leaf extracts have shown great potential for nano-synthesis [27]. Using endemic plants provides many advantages, such as easy accessibility, simplicity, economy, and ecological nature [28–31].

Some of the most important considerations of a green synthetic method are the use of nontoxic chemicals, benign solvents for the environment, and renewable materials [10, 32]. Besides, the process can be carried out at room temperature, and the reaction is completed in a few minutes. Green synthetic methods are simple, environmentally benign, and quite efficient for producing silver nanoparticles [10, 28].

6. Conclusion

We present a totally green approach toward the synthesis and stabilization of metal nanoparticles allowing to obtain an advanced suture that can be effective on oral and maxillofacial surgery, having demonstrated an antibacterial effect versus resistant bacteria. In this study, *Heterotheca inuloides* turns out to be an appropriate reducing agent for coating natural suture threads with AgNPs.

Synthesis of AgNp by eco-friendly reducing agents represents an environmental and economically sustainable process that minimizes the costs and provides the benefits and properties of plants such as *Heterotheca inuloides*.

We believe that this may be an alternative for surgeons, which helps in reducing the indiscriminate use of antibiotic therapy. It also represents an option to use advanced materials that are produced under sustainable conditions, which reduce the impact on the environment.

Conflict of interest

The authors declare that they do not have conflict of interest.

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