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# Antimicrobial activity of chitosan-silver nanoparticles made from jewelry industry silver waste

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**Abstract:** Local management of bacterial infections is challenging. The antimicrobial effect of silver has long been recognized, but its use is limited due to its expensive nature and reduced applicability in liquids. This study aimed to synthesize chitosan-silver nanoparticles (CS-AgNPs) from reusable silver waste of the jewelry industry and investigate their antimicrobial properties against pathogenic microorganisms. X-Ray diffraction (XRD) analysis was used to confirm the crystalline structure of the recycled silver, with a strong diffraction peak observed at $2\theta$=38.60°. Agar disk diffusion showed inhibitory effects for CS-AgNPs on the growth of *Escherichia coli*, *Staphylococcus aureus,* and *Candida albicans* that depended on the concentration of $AgNO_3$ solution used for preparation. In these tests, *S. aureus* was more susceptible to the treatment than *E. coli* and *C. albicans*. The CS-AgNP inhibited the growth of tested microorganisms with minimum inhibitory concentration ($MIC_{50}$) values between 1.7 and 4.25 mg/mL. These findings highlight the potential of CS-AgNPs as effective antimicrobial agents. The use of waste materials in nanoparticle synthesis in this research offers a promising approach for sustainable and eco-friendly nanotechnology.

**Keywords:** silver waste; jewelry industry; chitosan; nanoparticle synthesis; antimicrobial action

## INTRODUCTION

Bacterial infections are a major challenge because of the lack of new antibiotics and the spread of antibiotic resistance [1]. While the challenges of systemic antibiotics are more frequently discussed, topical antibacterial therapy faces similar issues [2]. Antibiotics are generally not suggested for local treatment due to their low efficacy (i.e., they do not reach bactericidal concentrations *in situ*), their probable contribution to the creation of resistant strains, and potential sensitization [3]. Consequently, there is a growing interest in exploring alternative antimicrobial strategies that are effective, safe, and environmentally friendly [4]. Antimicrobial agent research is particularly critical for surface disinfection, as demonstrated by the COVID-19 outbreak.

Chitosan is a commonly occurring natural polysaccharide. It is a biopolymer that has been applied for many purposes because it is biocompatible, biodegradable, non-poisonous, and has antimicrobial properties [5,6]. Chitosan possesses the ability to inhibit microorganism growth by interacting with its positively charged amino group and the negatively charged cell membrane, thus leading to the disruption of internal cellular components and proteinaceous constituents [7,8].

In recent years, researchers combined chitosan with metals to enhance its antimicrobial activity [9]. Among these metals, silver (Ag) has demonstrated greater antibacterial potential than other metals [10]. Silver ions ($Ag^+$) exert strong bactericidal effects and a broad range of antimicrobial activities. Silver nanoparticles (AgNPs) release potassium ions from bacteria and target parts of the cell such as the bacterial plasma or cytoplasmic membrane, which houses essential enzymes and nucleic acids [11]. The binding interaction between AgNP and chitosan results in the stabilization of the chitosan-AgNP (CS-AgNP) composites. Polymers are commonly used as stabilizers to prevent agglomeration and precipitation of particles [12]. As a result, when the composite is dissolved, nanoparticles attached to the polymer chains will disperse evenly in the solution [13].

The use of CS-AgNPs has potential in ophthalmology, wound treatment, orthopedics, and dentistry, either as localized antibacterial treatment or to prevent infections associated with joint replacements and dental restorations; AgNPs can significantly enhance the efficacy of microbial keratitis treatment [14]. Wound dressings containing AgNPs have generated widespread interest in wound healing, offering an economical option [5]. By providing fine structures for bone tissue engineering, nanoparticles in bone grafts have improved fracture repair partly due to their antimicrobial and possible osteoinductive capabilities [15]. AgNPs penetrate the root canal systems and dentinal tubules of teeth, while also improving the antibacterial qualities of endodontic irrigants and sealers. When employed as a carrier for intracanal medicines, AgNPs gradually raise dentin hardness in endodontically treated teeth [16].

The silver jewelry industry generates a substantial amount of silver waste during production and refining processes [17]. This waste is frequently sent to landfills, recycled, or repurposed to provide sustainable resources for other uses [18]. To date, several methods have been developed to facilitate the recycling process of silver from waste products [19]. However, limited information is available on using recycled silver for antimicrobial purposes.

This study utilized recycled silver obtained from jewelry industry waste to produce CS-AgNPs. The newly synthetized CS-AgNP composites were evaluated for their inhibitory effects against the growth of potential pathogens. Besides reducing the accumulation of silver waste in the environment, this is the first study to design an innovative way to reuse silver to find new antimicrobial agents.

## MATERIALS AND METHODS

### Ethics statement

The research study was conducted *in vitro* using bacterial and yeast cells with no human subjects or animals involved.

### Material preparation

The silver material used in this research was extracted from a liquid silver waste residue obtained from Kotagede Silver Industry (Indonesia). The $AgNO_3$ standard, $HNO_3$, formaldehyde (37%), $H_2SO_4$, acetone, and NaOH solutions were obtained from Sigma-Aldrich (Munich, Germany). The nutrient broth and agar, potato dextrose broth, and agar were purchased from Thermo Fisher Scientific (Waltham, MA, USA).

### Recycling of silver liquid waste

A 500 mL silver waste sample was added into a beaker, followed by 0.2 mol/L (37%) formaldehyde, and stirred until the solution became homogenous. Electroplating was performed using two nickel alloy plates (Sigma-Aldrich, Munich, Germany). The cathode and the anode were electrically connected with a voltage of 4 V, and the electroplating process was carried out for 1 h until silver particulate matter was obtained.

### X-ray diffraction analysis

The silver particles obtained after electroplating were dried for 12 h at 90±5°C. To attain a fine, uniform mixture, 1 g of a dry powder sample was ground with a mortar and pestle. The silver sample was examined by X-ray diffraction (XRD; using a XRD potentiometer, Bruker AXS, Billerica, MA, USA) with an acceleration voltage of 40 kV and a current of 44 mA in the $2\theta$ scan range of 3 to 80°, at a speed of 10.0 degree/min [20].

### Synthesis and characterization of CS-AgNP

The synthesis of $AgNO_3$ involved dissolving 0.85 g of recycled silver (Ag) in a solution of $NO_3$ in 100 mL of distilled water. This process yielded a concentration of 0.05 mol/L $AgNO_3$. The $AgNO_3$ solution was then diluted in distilled water to achieve concentrations of 0.03 mol/L and 0.01 mol/L. The 0.01 mol/L, 0.03 mol/L, and 0.05 mol/L $AgNO_3$ solutions were kept at room temperature until further use.

The synthesis of CS-AgNP was conducted according to Akmaz et al. [21] and Mirda et al. [22] with slight modifications. To generate a composite solution of CS-AgNPs, 0.1 g of chitosan was dissolved in 40 mL of 1% (v/v) acetic acid and stirred at room temperature using a magnetic stirrer at 250 rpm until complete dissolution. Subsequently, 2 mL of the 0.01 mol/L, 0.03 mol/L, or 0.05 mol/L $AgNO_3$ solutions were separately and gradually mixed into the chitosan solution, followed by stirring with a magnetic stirrer at 200 rpm. This process was carried out at 100 °C for 45 min. Afterward, 200 µL of NaOH (0.3 mol/L) was gently added until the color of the mixture turned yellow, about 1 min after the addition of NaOH, which indicated the formation of CS-AgNPs. CS-AgNP formation was monitored using a UV-Vis spectrophotometer (Shimadzu UV-2600i, Shimadzu, Duisburg, Germany). The absorption peak of CS-AgNPs was observed at 300 nm to 600 nm. The CS-AgNP samples were prepared using $AgNO_3$ concentrations of 0.01 mol/L, 0.03 mol/L, and 0.05 mol/L and designated as CS-AgNP1, CS-AgNP3, and CS-AgNP5, respectively.

### Antimicrobial activity of CS-AgNPs

*Escherichia coli* ATCC 11229, *Staphylococcus aureus* ETEC 0111 and *Candida albicans* ATCC 10231 microorganisms were applied to evaluate the antimicrobial effect of CS-AgNPs. To prepare fresh cultures for the experiments, one loop of the isolate from the corresponding agar slant was taken and inoculated to nutrient broth (in the case of *E. coli* and *S. aureus*) or potato dextrose broth (in the case of *C. albicans*) medium. The cultures were then incubated for 24 h at 37 °C.

### Agar disk-diffusion assay

The antimicrobial activity of CS-AgNPs on a solid medium was assessed by the agar disk-diffusion method [23]. One hundred µL of the prepared bacterial or yeast culture suspensions containing $10^5$ CFU/mL were evenly spread onto nutrient agar (for *E. coli* and *S. aureus*) or potato dextrose agar (for *C. albicans*) plates within Petri dishes. Following this, a volume of 20 µL of CS-AgNP at various concentrations was transferred to sterile paper disks, 5 mm in diameter, previously placed on the surface of the agar plate. Sterile paper disks containing 20 µL of distilled water or chitosan solution (2.5 mg/mL in 1% acetic acid) served as controls. The Petri dishes were placed in a temperature-controlled incubator at 37°C for 24 h. After the incubation period, the width of the inhibition zone around the disks was measured and the data were presented in mm.

### Minimum inhibitory concentration (MIC50) determination

The macrodilution method was applied to determine the $MIC_{50}$ value of CS-AgNP. Firstly, a 17 mg/mL stock solution was prepared from the nanoparticle concentrate in the medium used for cultivation. The CS-AgNP stock solution was mixed in 1 mL of nutrient broth (for *E. coli* and *S. aureus*) or potato dextrose broth (for *C. albicans*) to reach testing concentrations of 8.50, 7.65, 6.80, 5.95, 5.10, 4.25, 3.40, 2.55, and 1.70 mg/mL. After inoculation ($10^5$ CFU/mL), the cultures were incubated under continuous shaking at 37°C for 24 h. Optical density (600 nm) measurement was performed to monitor the relative growth of the microorganisms using a Shimadzu UV-2600i spectrophotometer (Shimadzu, Duisburg, Germany). The concentration of the CS-AgNP that caused 50% or higher growth inhibition was considered the $MIC_{50}$. The control in this experiment consisted of an inoculated growth medium without the addition of CS-AgNP.

### Effect of CS-AgNPs on microbial growth during prolonged incubation

The effect of CS-AgNP was also monitored during 48 h of incubation. The $10^5$ CFU/mL microbial suspensions were inoculated into the corresponding liquid medium containing CS-AgNP samples prepared with three

different $AgNO_3$ concentrations, 0.01 mol/L, 0.03 mol/L, and 0.05 mol/L. The control in this experiment consisted of an inoculated growth medium without the addition of CS-AgNPs. The cultures were then incubated at 37°C for 48 h. The optical density was measured every three hours at a wavelength of 600 nm (Shimadzu UV-2600i spectrophotometer, Shimadzu, Duisburg, Germany).

### Statistical analysis

Assays were performed in three independent parallel experiments, and data were expressed as the means±standard deviation. Statistical analysis of the data was carried out using one-way analysis of variance (ANOVA) (SPSS version 22.0, IBM Corporation, New York, USA), and Duncan's multiple range test was used to determine the significant variation ($P<0.05$).

## RESULTS

### XRD analysis of silver sample

The XRD analysis of the recycled silver sample displayed diffraction peaks at $2\theta$ values of 24.38°, 29.88°, 31.03°, 32.67°, 33.84°, 36.64°, 38.60°, 44.78°, 60.02°, and 64.88° (Fig. 1). The crystalline structure of the recycled silver was confirmed by the observed XRD patterns, with a prominent diffraction peak detected at $2\theta = 38.60°$.

### Characterization of CS-AgNPs

According to the UV-Vis spectrophotometry results (Fig. 2), maximum absorbance of the synthesized CS-AgNPs was between 390 nm and 430 nm. The CS-AgNP5 with a silver concentration of 0.05 mol/L exhibited a shorter wavelength at maximum absorbance than CS-AgNP3 (0.03 mol/L silver concentration), and CS-AgNP1 (0.01 mol/L).

### Antimicrobial effect of CS-AgNPs in agar disk-diffusion tests

The antimicrobial activity of CS-AgNPs prepared from the reusable silver waste was investigated against *E. coli*, *S. aureus*, and *C. albicans* by agar disk-diffusion test. The sensitivity testing of microorganisms using agar diffusion in zones of inhibition are shown in Table 1.

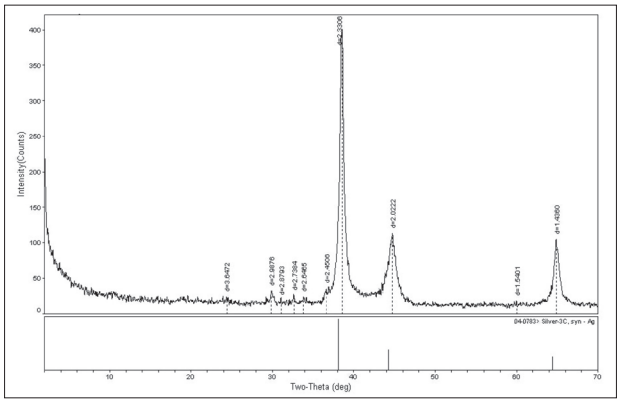


**Fig. 1.** X-ray diffraction (XRD) pattern of recycled silver.

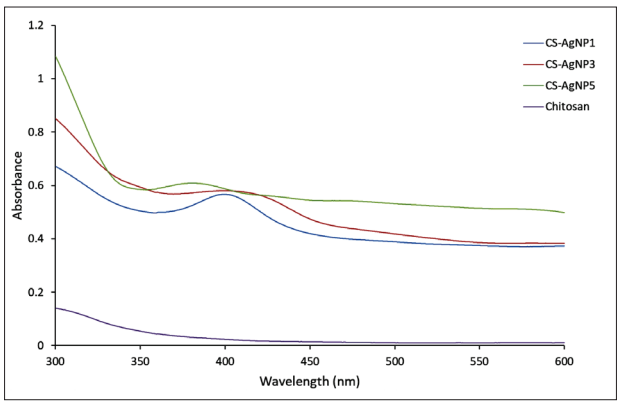


**Fig. 2.** Comparison of the UV-Vis results of CS-AgNP1, CS-AgNP3, CS-AgNP5, and pure chitosan samples. CS-AgNP1, CS-AgNP3, and CS-AgNP5 particles were prepared using $AgNO_3$ at concentrations of 0.01 mol/L, 0.03 mol/L, and 0.05 mol/L, respectively.

**Table 1.** Antimicrobial activity (in mm zone of inhibition) of different concentrations of CS-AgNPs against *E. coli*, *S. aureus*, and *C. albicans* after 24 h incubation. CS-AgNP1, CS-AgNP3 and CS-AgNP5 particles were prepared using $AgNO_3$ at concentrations of 0.01 mol/L, 0.03 mol/L and 0.05 mol/L, respectively.

| CS-AgNP materials | Growth inhibition (mm) | | |
|---|---|---|---|
| | *Escherichia coli* | *Staphylococcus aureus* | *Candida albicans* |
| Distilled water control | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| Chitosan control (2.5 mg/mL) | 2.19±0.10$^{b}$ | 8.62±0.46$^{b}$ | 3.61±0.31$^{bc}$ |
| CS-AgNP1 | 3.54±1.05$^{c}$ | 7.75±0.17$^{c}$ | 3.15±0.37$^{b}$ |
| CS-AgNP3 | 4.42±0.43$^{c}$ | 9.96±0.68$^{d}$ | 3.05±0.87$^{b}$ |
| CS-AgNP5 | 4.32±0.39$^{c}$ | 7.83±0.49$^{bc}$ | 4.87±1.28$^{c}$ |

Different letters within a column represent significant differences ($P<0.05$) compared to the matching distilled water control (one-way ANOVA, Duncan's multiple range test). Values shown are the means±SD.

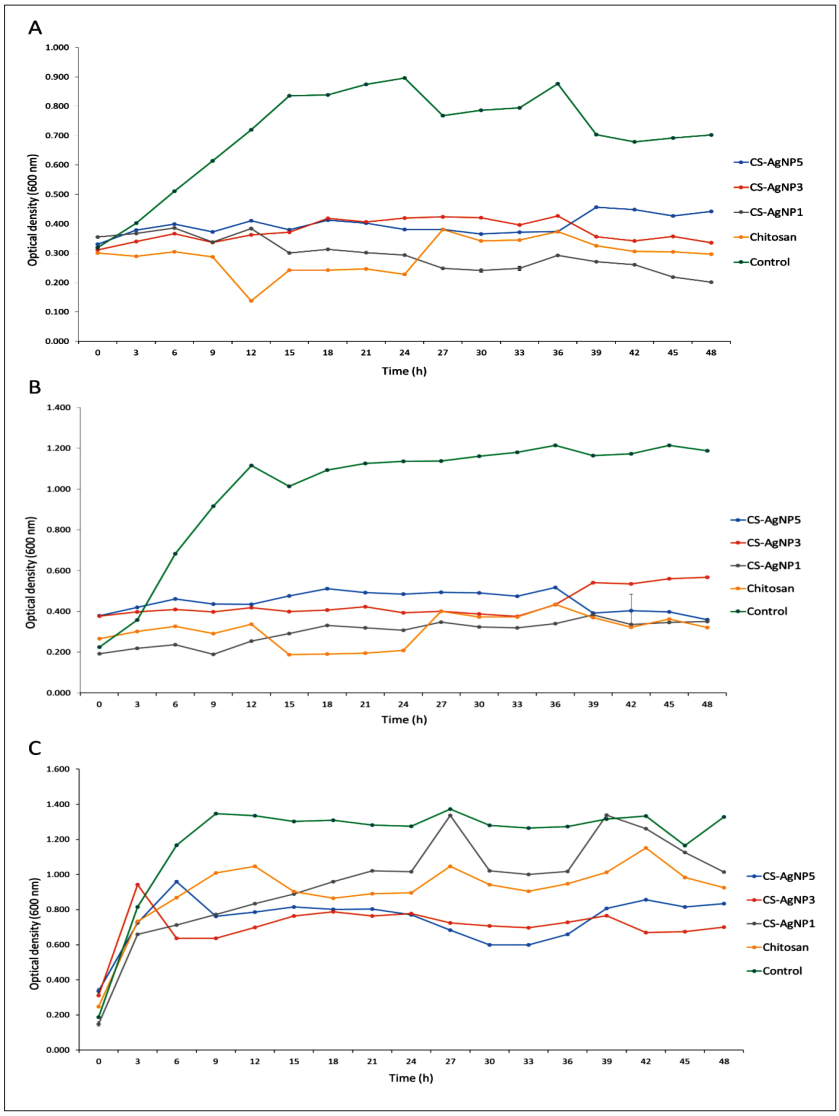


**Fig. 3.** Effect of CS-AgNPs (0.01 mol/L, CS-AgNP1; 0.03 mol/L, CS-AgNP3; 0.05 mol/L, CS-AgNP5) on the growth of *E. coli* (**A**), *S. aureus* (**B**), and *C. albicans* (**C**) during 48 h of incubation. CS-AgNP1, CS-AgNP3, and CS-AgNP5 particles were prepared using $AgNO_3$ at concentrations of 0.01 mol/L, 0.03 mol/L and 0.05 mol/L, respectively.

CS-AgNPs in the medium prevented the growth of bacteria and yeast, as indicated by the inhibition zones observed. CS-AgNP1, CS-AgNP3, and CS-AgNP5 exhibited higher inhibition compared to the chitosan control in *E. coli*. The inhibitory effects of the CS-AgNP3 and CS-AgNP5 samples were greater than those of the chitosan control in *S. aureus* and *C. albicans*. The inhibition zones presented by these CS-AgNP samples ranged from 3.54 mm to 9.96 mm and showed a significant difference ($P<0.05$) compared to the inhibitory zone of the chitosan control. The results demonstrated that higher concentrations of silver generally led to larger inhibition zones. In addition, *S. aureus* and *E. coli* were more susceptible to the tested CS-AgNPs than *C. albicans*.

## Minimum inhibitory concentration (MIC50) of CS-AgNP

The $MIC_{50}$ of CS-AgNP5 was determined following a 24-hour exposure period. Distinct $MIC_{50}$ values for the CS-AgNP were noted. The $MIC_{50}$ of the CS-AgNP5 sample for *E. coli* and *S. aureus* was 1.70 mg/mL, while the $MIC_{50}$ for *C. albicans* was 4.25 mg/mL. The variation in MIC values highlighted the potent antimicrobial efficacy of CS-AgNPs based on the microbial strains, suggesting their potential for diverse biomedical and pharmaceutical applications.

## Effect of CS-AgNPs on microbial growth during prolonged incubation

The effect of CS-AgNPs on microbial growth in the liquid medium was confirmed, and the presence of CS-AgNPs resulted in lower growth than in the control tubes, with different growth patterns for the tested microorganisms in the presence of CS-AgNPs. During the first 6 h of incubation, Gram-negative *E. coli* was more susceptible than Gram-positive *S. aureus* (Fig. 3A and B). Furthermore, OD measurements demonstrated that CS-AgNPs resulted in lower values compared to the control for *C. albicans* (Fig. 3C). The highest reduction in the growth of *E. coli* and *S. aureus* was identified in the presence of CS-AgNP1, and for *C. albicans*, the CS-AgNP3 and CS-AgNP5 samples exhibited the highest inhibitory effect.

## DISCUSSION

The presence of dissolved inorganic materials in industrial liquid waste is a significant environmental concern due to the potential leaching of toxic compounds into the environment and the loss of valuable metals that remain in the waste stream [18]. The recovery of metals from waste is essential for their continued use. This study successfully employed recycled silver extracted from silver jewelry industrial liquid waste to synthesize nanoparticles for antimicrobial properties. XRD characterization indicated a sharp peak at 38.75° from the silver sample that was also observed previously [24], confirming the presence of silver nanoparticles. The recycled silver thus could be used as a critical component for subsequent CS-AgNP synthesis, consistent with other studies [20]. The range of the absorption spectrum for the samples was predominantly around 400 nm, which is consistent with previous reports indicating characteristic peaks of AgNPs in the range of 400-450 nm [5,25]. This result suggests that the CS-AgNP synthesis process is influenced by the Ag concentration, leading to variations in the properties and antimicrobial potential of the nanoparticles.

The antimicrobial activity demonstrated variations in the inhibition zones, as reflected by the differences in clear zones surrounding the microbial colonies across the tested microorganisms. AgNPs have been known to have considerable antimicrobial effects against both bacteria and yeast [26]. Our findings revealed that *S. aureus* is more susceptible to CS-AgNPs than *E. coli*, which aligns with the study of Gomaa [27] that noted *S. aureus* is more sensitive than *E. coli*.

Our results highlight the influence of chitosan, an additional component in CS-AgNP, which may contribute to these observed differences. Chitosan, as a polymer stabilizer, plays a crucial role in preventing nanoparticle aggregation and improving biocompatibility properties [28]. It acts as a matrix material for the growth and stability of nanoparticles, effectively immobilizing metal nanoparticles within a polymer matrix [29]. Structurally, chitosan possesses amine ($-NH_2$) and hydroxyl (-OH) functional groups, which act as chelating and reducing agents in the synthesis of silver nanoparticles while also preventing their agglomeration [30,31]. These properties, combined with its high viscosity, antimicrobial activity, biodegradability, non-toxicity, safety, and eco-friendliness, make chitosan an excellent stabilizing agent in nanoparticle synthesis [32,33].

The $MIC_{50}$ results showed that *E. coli* and *S. aureus* had lower $MIC_{50}$ values than *C. albicans*, suggesting that prokaryotic cells can be more sensitive than eukaryotic cells, consistent with previous research [34]. The MIC values identified are consistent with a prior study [35], which revealed a 1.25 mg/mL MIC of AgNPs mediated with *Strobilanthes crispus* water extract towards *E. coli*, *Pseudomonas aeruginosa*, and *Streptococcus mutans*. Sanchooli et al. [36] found the MICs of AgNPs to be 2.5, 0.32, and 1.25 mg/mL against *Vibrio cholerae*, *Yersinia ruckeri*, and *Listeria monocytogenes*, respectively.

The effect of the three CS-AgNP samples on microbial growth in liquid medium over a 48-h incubation period exhibited distinct patterns for each strain. The CS-AgNP1 prepared with 0.01 mol/L $AgNO_3$ exhibited greater effectiveness in inhibiting the growth of *E. coli* and *S. aureus*. This increased antimicrobial effectiveness for CS-AgNP prepared with the lowest $AgNO_3$ concentration could be attributed to the thinner peptidoglycan layer in Gram-negative bacteria, which renders them more susceptible to radical damage [37]. Conversely, in the Gram-positive bacteria, the thicker peptidoglycan layer becomes more accessible, facilitating the permeation of silver nanoparticles, which allows the nanoparticles to easily penetrate and disrupt the integrity of bacterial cell walls [38]. CS-AgNPs also exhibit an adverse impact on the physiological and biochemical processes of *C. albicans* [39].

Chitosan, as an additional component in the nanocomposite, may contribute to this enhanced antimicrobial activity. It possesses inherent antimicrobial properties and can disrupt the growth and viability of bacterial cells. A previous study showed that colloidal AgNP inhibits *S. aureus* to a greater extent than *E. coli* [40], suggesting variations in the antimicrobial effects depending on the bacterial species. The mechanism of action of AgNPs in *S. aureus* involves bactericidal activity and multiple biological pathways through the destruction of key proteins [41].

Moreover, chitosan (0.01%) has been reported to inhibit the growth of spoilage bacteria such as *E. coli* and *S. aureus* in a liquid medium [42]. As expected,

the antibacterial activity against the Gram-positive *S. aureus* of chitosan alone was lower than the CS-AgNP [43]. Chitosan oligosaccharide can sensitize multidrug-resistant *S. aureus* to antibiotic formulations by interacting with multidrug efflux pumps through electrostatic interactions [44]. Furthermore, chitosan and chitosan nanoparticles display antimicrobial activity against gastrointestinal pathogens including *E. coli* [45]. The CS-AgNP5 (prepared with 0.05 mol/L $AgNO_3$) demonstrated a strong inhibitory effect against *C. albicans*, which is a common fungus in the healthy human gut [46]. The fungicidal activity of CS-AgNP is positively associated with the degree of acetylation and negatively related to molar weight [47]. These results indicate that the antimicrobial activity of CS-AgNP nanoparticles varies depending on the targeted microorganism, and the physicochemical characteristics of the inhibitory agent.

The evaluation of antimicrobial activity revealed variations in the inhibition zones among different microorganisms, potentially attributed to the presence of chitosan in the nanoparticles [48]. The concentration-dependent effect of CS-AgNP on microbial growth highlighted the *C. albicans* response, enhancing our understanding of CS-AgNPs' antimicrobial properties and potential for combating pathogenic microorganisms. However, this investigation focused on a limited range of CS-AgNP concentrations, potentially limiting the dose-dependent effects.

AgNPs have attracted considerable attention for their use in biomedical applications. However, despite their numerous beneficial properties, their biomedical potential is limited by the low stability of uncoated AgNPs and their high toxicity. Therefore, chitosan aids in improving the colloidal stability of Ag-based nanomaterials, as particle aggregation can undermine their therapeutic effectiveness [49].

In this study, the quantity of waste utilized for producing AgNPs is a critical aspect meriting deeper exploration. The use of waste materials in nanoparticle synthesis offers a promising approach for sustainable and eco-friendly nanotechnology. Our findings suggest that the selected waste amount was adequate to yield nanoparticles with desirable antimicrobial properties, indicating that the process was efficient. Our current methodology does not require large quantities of waste. This study contributes to the growing field of green nanotechnology and highlights the potential of waste-derived materials in advancing sustainable industrial practices.

## CONCLUSIONS

CS-AgNPs were synthesized using a silver waste substance from the jewelry industry, and the produced nanoparticles were characterized according to their structural and antimicrobial properties. XRD analysis confirmed the crystalline structure of the silver. The absorbance peak indicated a relationship between Ag concentration and the quantity of CS-AgNP materials produced. Agar diffusion tests showed CS-AgNP antimicrobial activity against *E. coli*, *S. aureus*, and *C. albicans*. The CS-AgNPs were more effective against the bacteria *S. aureus* and *E. coli* than the yeast *C. albicans*. In prolonged incubation tests up to 48 h, the presence of CS-AgNPs in the medium significantly reduced microbial growth compared to the control. The synthesized CS-AgNPs from reusable silver waste showed promising potential as alternative antimicrobial agents. Future research should explore the mechanism of action, optimize the CS-AgNP formulation, and conduct *in vivo* studies to validate their efficacy as antimicrobial agents.

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**Conflict of interest disclosure:** The authors declare no conflict of interest.

**Data availability**: Data underlying the reported findings have been provided as a raw dataset available here: https://www.serbiosoc.org.rs/NewUploads/Uploads/Perdana%20et%20al_10273_Dataset.xlsx

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