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Tomatoes By-Products Extracts Mediated Green Synthesis of Silver Nanoparticles and Their Application as Antimicrobial Agent

Aistė Balčiūnaitienė, Jonas Viškelis, Dalia Urbonavičienė and Pranas Viškelis

Abstract

Silver nanoparticles (AgNPs) biosynthesized using by-products of tomatoes extracts as reducing and capping agents show multiple possibilities for solving various biological problems. The aim of this study was to expand the boundaries on AgNPs using novel low toxicity and production cost phytochemical method for the biosynthesis of nanoparticles from tomatoes aqueous extracts. Biosynthesized AgNPs were characterized by various methods (SEM, EDS). Determined antioxidative and antimicrobial activity of plant extracts was compared with the activity of the AgNPs. TEM results show mainly spherical-shaped AgNPs, size distribution of which depends on the plant leaf extract type; the smaller AgNPs were obtained with tomatoes extract (6–45 nm AgNPs). Besides, AgNPs show strong antimicrobial activity against broad spectrum of Gram-negative and Gram-positive bacteria strains and fungi.

Keywords: silver nanoparticles, green synthesis, tomatoes by-products, antimicrobial activity

1. Introduction

There are various bacterial, fungal, viral, and other microscopic life forms in our environment. Microorganisms make up 80–90% of the earth's total biomass, and even under “clean” conditions, several thousand fungal spores can be inhaled per day. Many microorganisms are harmless or even beneficial, but others can be extremely dangerous or even deadly. The current way of life creates favorable conditions for the spread of infections (food from distant lands, work in air-conditioned rooms, frequent trips to foreign countries, visits to hospitals, etc.). The human body is not sterile, and it is colonized by many microorganisms that are part of the normal microflora and live like harmless commandants.

Microorganisms living under normal conditions on the skin, nasopharynx, and intestine play an important protective role, as they prevent the growth of pathogenic

microorganisms in these places. As the bodies condition changes (weakened immunity, disease, or trauma), the so-called non-pathogenic bacteria can become pathogenic and cause infections. Wounds are susceptible to contamination by microorganisms both externally and from internal sources in the body, such as the nasopharynx, skin, and gastrointestinal tract. Infection is the result of a constantly changing interaction between microorganisms, the human being as their host, and the environment around them. Exposure of the Gram-positive and Gram-negative bacteria strains to the host's defense capacity interferes with wound healing and potentially dangerous changes in the body due to infection.

According to research, more than 23,000 people die each year in Europe from invasive (or systemic) infections caused by *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). It has also been observed that these infections are increasing rapidly due to the progressive, excessive use of antimicrobials, which allows pathogenic microorganisms to evolve and acquire multiple antibiotic resistance. Therefore, scientists around the world are constantly looking for new ways and materials to combat the colonization of pathogenic microorganisms [1]. Thus, the problems associated with unwanted bacterial adhesion to the surfaces of medical equipment, as well as the colonization of surgical equipment, implants, and other health-related products, pose a significant risk to public health. The formation of biofilms also directly affects many industrial processes: food processing and storage, water treatment processes, maritime transport and management. Various antimicrobial agents are commonly used against biofilms and their infections, but microbiological control of the process is hampered by the ability of pathogenic microorganisms to attenuate or acquire full resistance to antimicrobial compounds, including antibiotics. Despite ongoing efforts by scientists to avoid bio-contamination and additional control measures implemented by industry, there is still no effective solution to protect the surface of equipment from colonization by pathogenic microorganisms. For these reasons, the need for antimicrobials is greater than ever before.

2. Antimicrobial agents

The increasing level of pollution by microorganisms and infections creates the need for new antimicrobial agents. Therefore, the research on the development and application of polymer composites with antimicrobial activity is of great interest.

Plant-mediated synthesis imparts several advantages to metal nanoparticles (MNPs) technology for the development of alternative products against infectious diseases. Indeed, most of green MNPs from plant-derived materials are highly effective and nonspecific antimicrobial agents, showing remarkable activities against the growth of a broad spectrum of bacterial and fungal species, in both planktonic and biofilm forms, including nosocomial and multidrug-resistant strains [2, 3].

Materials with antimicrobial activity are abundant. One of the largest groups is natural or synthetic antibiotics, which inhibits the appropriate stage of synthesis of the microorganism's cellular proteins. However, excessive use of antibiotics has led to the emergence of strains of bacteria that are resistant to most antibiotics, posing a significant risk to public health. As a result, other substances with antimicrobial activity are increasingly being used. Their nature can be very different. These are, in particular, substances of natural origin: vegetable (various essential oils, medicinal plant extracts, etc.) and animal (e.g., lysozyme, lactoferrin), microbes (nisin, nistatin, etc.), as well as inorganic and organic synthetic and hybrid derivatives of a

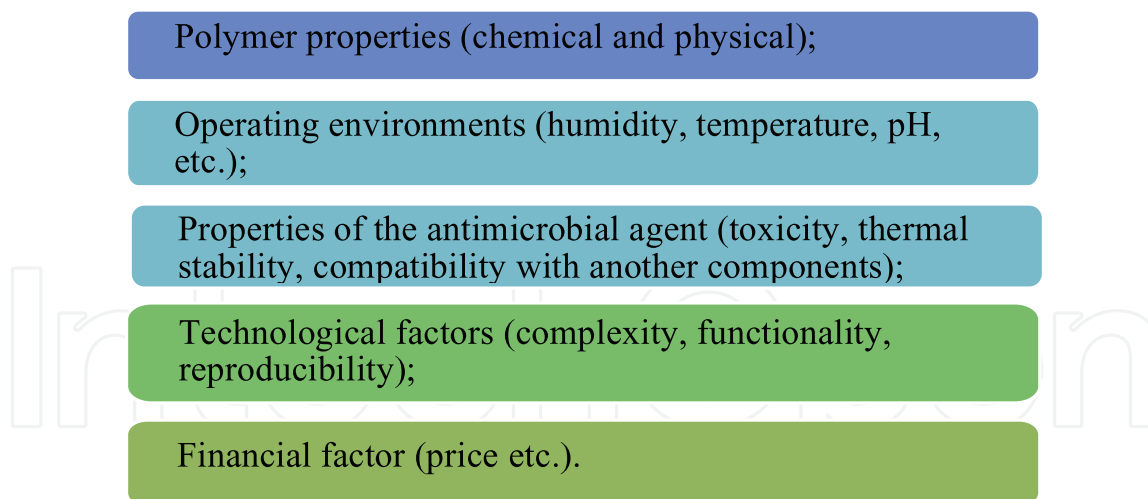


Figure 1.
The choice of antimicrobial modification method.

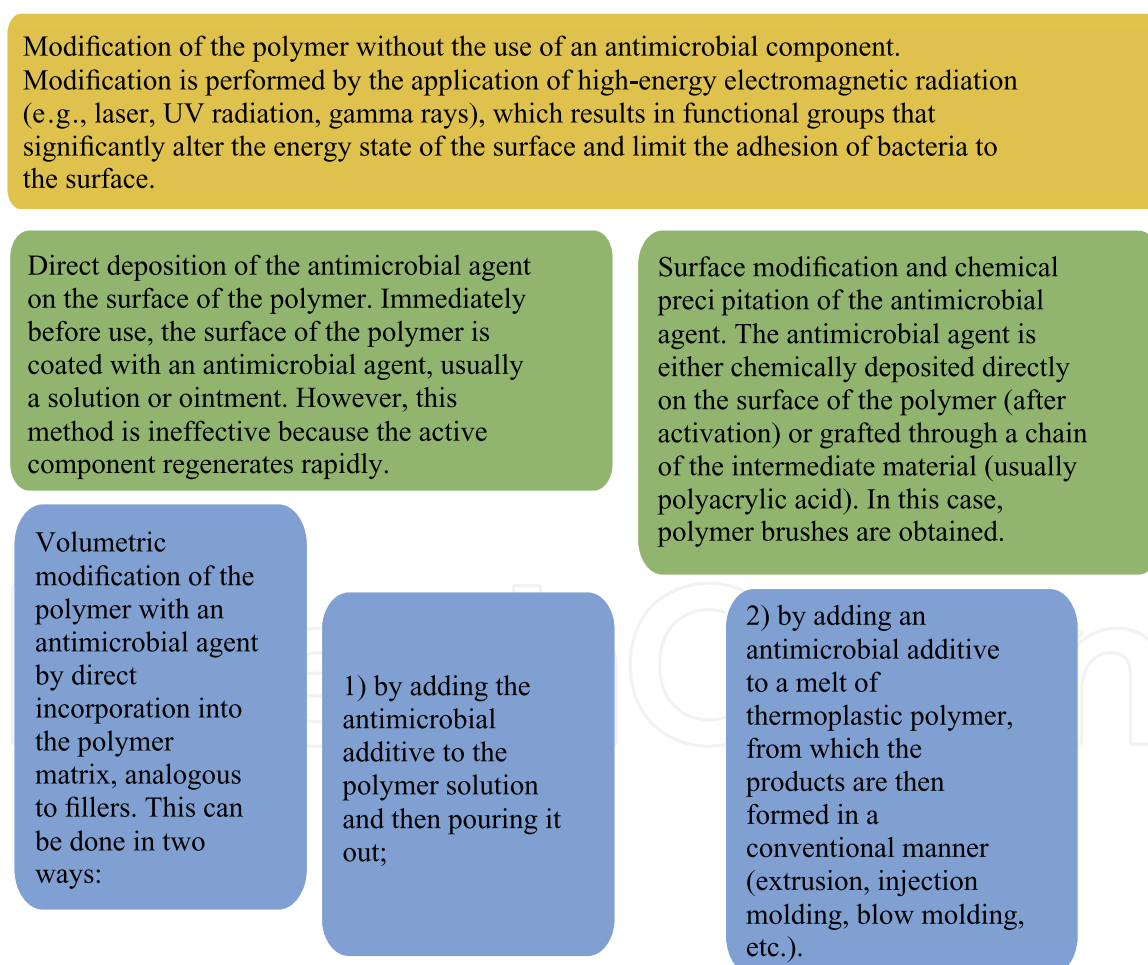


Figure 2.
The main methods of antimicrobial modification of polymers [4].

nature. Polymeric materials that are resistant to the colonization of microorganisms and the spread and multiplication of pathogenic microorganisms are also one of the groups of antimicrobials. They usually consist of a polymeric matrix and an embedded antimicrobial component.

The choice of antimicrobial modification methods depends on many factors (Figure 1).

The main methods of antimicrobial modification of polymers are as presented in Figure 2.

3. Antimicrobial activity of metal nanoparticles

One of the most abundant groups of substances with antimicrobial activity suitable for polymer modification is inorganic compounds and metal nanoparticles. This group consists of metals (Ag, Au, Cu, etc.), metal oxides (ZnO, TiO₂, etc.) [5], nonmetallic oxides (SiO₂). In most cases, the size of antimicrobial nanomaterials ranges from 1 to 100 nm. They can be of organic or inorganic origin, but inorganic substances are most commonly used. Nanoparticles are the most widely used because they have broad-spectrum antibacterial properties against both Gram-negative and Gram-positive bacterial strains [6]. The main reason why nanoparticles are an alternative to antibiotics is their ability to inhibit multiresistant microorganisms in some cases. Nanoparticles have a large surface area that increases interaction with microorganisms, resulting in strong antimicrobial activity. Nanoparticles with a smaller size and a higher surface area to weight ratio are more efficient at breaking biofilms. The particle shape also has a significant effect on the degradation efficiency of biofilms (e.g., rod-shaped particles are much more efficient than spherical forms). There are various methods for the synthesis of nanoparticles, which can be divided into two main classes: (1) bottom-up, and (2) from top to bottom [7].

In general, the chemical, physical, mechanical, and antimicrobial properties of nanoparticles depend on their chosen precursor. Nanoparticle microorganisms act in different ways, and the mechanism of their action depends on the origin of the nanoparticle [8]. Nanoparticles have antibacterial (inhibits DNA replication, enzyme functions, etc.), antiviral (blocks the attachment of viruses to the cell wall), antifungal (breaks down the cell membrane), and other effects.

Nanotechnology is the science, engineering, and technology that studies matter at the atomic, molecular, or supramolecular levels to yield nanometric materials and nanosystems with improved properties such as high surface-to-volume ratio and high dispersion in solution. With size typically ranging between 1 and 100 nm, these nanomaterials and nanosystems can be synthesized by chemical, physical, and/or biological methods [9]. In comparison with chemical and physical methods that involve costly and toxic chemicals, the biological synthesis pathway based on the usage of biological sources (plants, bacteria, fungi, and algae) is hoisted as a real rescue route. In spite of that, the biological methods do not envisage the use of toxic catalysts and reagents, dealing exceptionally with the intracellularly or extracellularly produced metabolites within fermentation routes, and this method requires big input of costly materials, well-developed protocols and guidelines, and microbiological hands-on experience to ensure cell culture and nanoparticles purification under highly aseptic conditions.

In contrast, the use of plant-derived extracts, juice, hydrolysates, etc., for the biosynthesis of metal nanoparticles (MNPs) seems to be an environmentally friendly, cost-effective, robust, sustainable alternative with moderate reaction conditions [10]. The plant-mediated synthesis of nanoparticles is also biocompatible, clinically adaptable, and easily up-scalable for industrial production [11]. Plants could represent

continuous source of natural antioxidants and antimicrobials (polyphenols, flavonoids, tannins, terpenoids, alkaloids, essential oils, etc.) suitable for green synthesis of nanoparticles with desirable properties. Under proper extraction conditions dealing with nontoxic organic solvents, diverse spectrum of non-deleterious reducing agents could be acquired [12].

Recent evidence in the field of nanotechnology revealed that the morphological parameters of nanoparticles (e.g., size and shape) can be modulated by varying the concentrations of bioactive compounds and reaction conditions (e.g., temperature and pH). Due to multiple therapeutic and biological activities such as antioxidative, antimicrobial, anti-inflammatory, anticancer, eugenol as a representative of phenylpropanoids received tremendous interest among researchers. The crude extracts recovered from such herbal plants as Lamiaceae, Lauraceae, Myrtaceae, and Myristicaceae, the major compound of which was eugenol, have been investigated in terms of reducing ability for nanoparticles synthesis. However, less explored are other sources of this unique molecule, especially by-products that also could provide adequate quantities of eugenol. Considering the evidence on the presence of eugenol, a principal component of lignin in cereals and their by-products (bran), and already established protocol for lignocellulose biomass hydrolysis, it is speculated that the process of biorefining could represent a sustainable and reliable way of bran utilization for the production of eugenol-based nanoparticles, thereby contributing to waste reduction. Additionally, using different sources of metals (salts or oxide) in combination with plants, the biological reduction method allows the synthesis of a large number of green MNPs, including silver (Ag), gold (Au), zinc oxide (ZnO), platinum (Pt), palladium (Pd), copper (Cu), iron oxide (Fe_2O_3 and Fe_3O_4), nickel oxide (NiO), magnesium oxide (MgO), titanium dioxide (TiO_2), and indium oxide (In_2O_3).

Considering the above, exploration of the plant systems as potential bio-factories for MNPs has gained considerable attention, especially for researchers working in the field of phytonanotechnology, pharmaceutical, and clinical microbiology as well as medicine [7]. Indeed, due to the surging popularity of green methods, more than 2000 research papers and reviews related to antibacterial, antifungal, and antibiofilm properties of MNPs have been published. Noteworthy, most of the reviews and research articles published so far focused mainly on predicting the antimicrobial mechanisms of MNPs and parameters that may influence their antibacterial, antifungal, and activities such as.

The type (and origin) of plants used as bioreactor sources for biosynthesis;

The reduction process of the metal salts (mainly silver, zinc and gold) used during the bio-fabrication of nanoparticles;

The particulate characteristics of MNPs (size, zeta potential and shape) as well as the characterization techniques allowing their determination;

The general protocols.

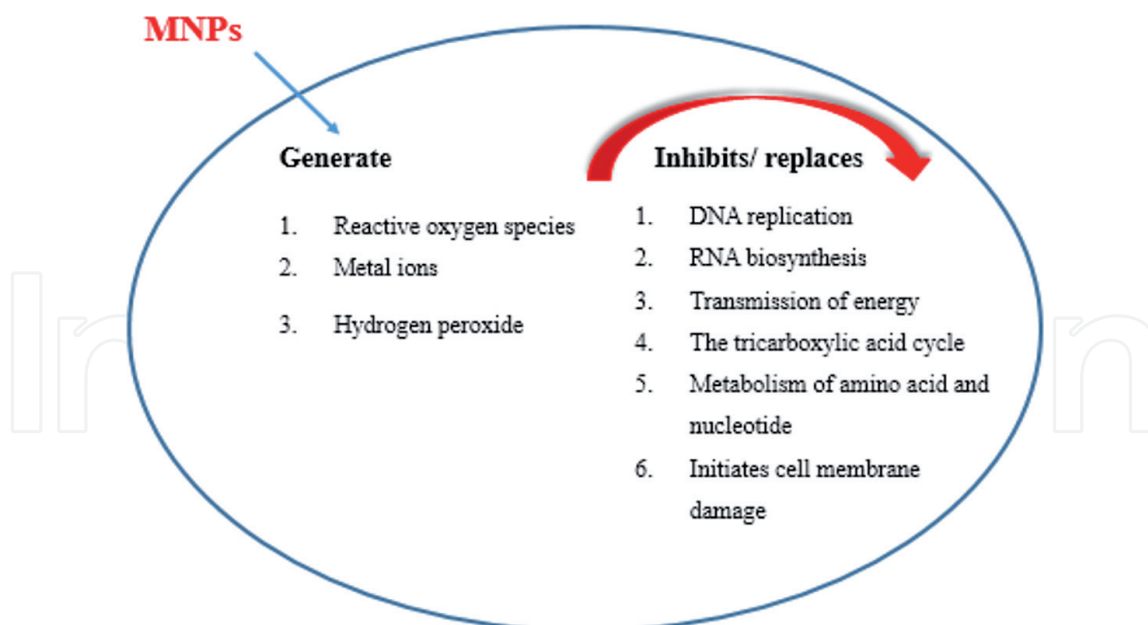


Figure 3.
Mechanisms of antimicrobial action of nanoparticles on bacterial cells.

Unfortunately, it appears from these reviews that the methods used for assessing the antibacterial, antifungal, and antibiofilm efficiency of MNPs are only partially elaborated in terms of standardization process; therefore, it is hard to correlate or compare data from different studies to pinpoint the high values of antimicrobial nanoparticles. Moreover, such methodologies and models are usually hard to extrapolate to real products.

Metal nanoparticles can affect the bacterium even in several ways, causing extremely strong antimicrobial activity (**Figure 3**). The most common and widely used silver nanoparticles, elemental silver has been widely used as an antimicrobial agent since ancient times. To improve their antibacterial activity and reduce their toxicity, silver ions can be transformed into metallic silver nanoparticles through biological and biomimetic methods of synthesis. Green AgNPs have demonstrated the ability to reduce microbial infections in the skin and burn wounds and prevent bacterial colonization on the surface of various medical devices such as catheters and prostheses. Acting as capping agents, different multifunctional phytochemicals contribute efficiently to these antimicrobial activities [8]. Moreover, AgNPs can express synergism with standard antibiotics such as gentamycin and streptomycin [13]. Hence, these combinations can effectively be used against antibiotic-resistant pathogens. Additionally, antifungal activities of AgNPs have extensively been studied and demonstrated in the literature [14]. In the frame of the fight against antibiotic resistance, green synthesized AgNPs may be used as vehicles to transport oligonucleotide-based antimicrobial. Their synthesis can be performed by physical, chemical, or biological methods. Particle size, morphology, and antimicrobial activity differ according to the chosen method [15].

Numerous studies have shown that silver nanoparticles, in both colloidal and ionic forms, have a broader spectrum of antibacterial activity than most other nanoparticles. Due to their unique optical, electrical, and chemical properties, silver nanostructures are widely used in a variety of industries. However, they are most commonly used in health care and medicine due to their strong antimicrobial activity against many pathogenic microorganisms—Gram-positive, Gram-negative, and antibiotic-resistant bacterial species, fungi, and viruses.

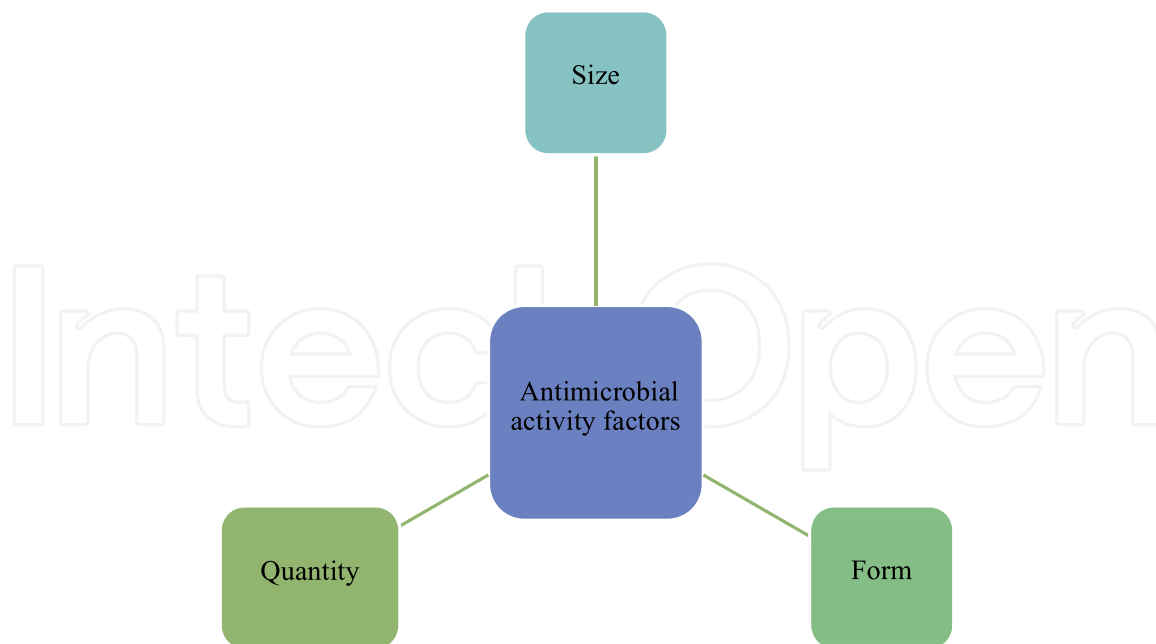


Figure 4.
Factors determining the antimicrobial activity of silver nanoparticles.

Rapid wound healing is due to decreased matrix metalloproteinase and increased neutrophil apoptosis in the wound induced by silver compounds. Matrix metalloproteinase is thought to be able to initiate inflammation and thus slow wound healing, so its regulation is very important [16]. Silver nanoparticles are also used in bone cement in various disinfectants [13] as antimicrobial agents. Beside this, their effect depends on several factors presented in the figure (**Figure 4**).

Antimicrobial activity of silver ions is obtained by reacting with the main components of the bacterium:

- cell wall and plasma membrane,
- DNA and proteins.

Due to the small size and very large specific surface area, silver nanoparticles adhere firmly to the surface of the bacterium. Silver ions, by interacting with the bacterial cell membrane and the sulfur compounds present in its proteins, impair its functionality. Further, silver nanoparticles penetrate the cell and damage DNA. Silver ions react with phosphorus compounds present in DNA, disrupting the process of DNA replication, which inhibits bacterial proliferation. They also degrade bacterial proteins, especially enzymes that catalyze metabolic reactions and other vital cellular processes. In addition, nanoparticles lead to the formation of reactive oxygen species, which are active and unstable molecules that can damage cellular DNA, protein structures, and cell membranes [17].

The antimicrobial activity of silver ions in Gram-positive and Gram-negative bacterial cultures may be different due to differences in bacterial cell structure. The cell wall of Gram-positive and Gram-negative bacteria has a complex, semi-rigid structure. The structure of the wall is very important because it determines the ability of the bacteria to cause disease and resistance to certain antibiotics. The wall thickness of the bacterial cells is unequal. The cell wall of Gram-positive prokaryotes is composed of a network of macromolecules called peptidoglycan or murein,

polysaccharides, lipids, and proteins. The wall thickness is much higher (20–80 nm) than that of Gram-negative bacteria. Their prokaryotic cell wall is composed of several layers: The inner dense electron layer (2–3 nm) is composed of peptidoglycan, two dense electron bands separated by an electron-conducting cavity, a space separated by the periplasmic cavity of the cytoplasmic membrane. The cell wall of Gram-positive microorganisms adheres closely to the cytoplasmic membrane [18]. These differences between bacterial species lead to unequal interactions between antimicrobial compounds. It is clear that metal nanoparticles are promising as antimicrobial agents and therapeutic agents due to their biological, physical, and chemical properties. They can solve many problems in the field of nanomedicine. However, there is a lack of knowledge about the long-term effects of nanoparticles on human health and the environment. Nanoparticles are stable and can accumulate in the environment; they have a tendency to agglomerate and can therefore change their dimensions. Toxicity studies of nanoparticles have shown that metal nanoparticles can act at the organ, tissue, cell, muscle, and protein levels. Nanoparticles are extremely small in size and can easily spread through air or water and adversely affect the skin, lungs, and brain (especially nanoparticles with dimensions 10 nm).

Therefore, the search for other substances with antimicrobial activity, such as the use of plant-derived substances to obtain antimicrobial compounds, is intensifying [19].

4. Morphology and antimicrobial activity of tomatoes by-products and green AgNPs

The aim was to compare the morphological differences of tomato pulp by variety. From **Figure 5**, the micrographs presented by SEM can show that the tomato particles are irregular in shape, with an uneven, layered surface, and that the particles appear to be composed of discrete slender shapes without any visible particles on the surface. The average particle diameter is very uneven as it was not fractionated, but the particle size could be harmonized by choosing milling techniques and conditions. Also, the particle size may vary depending on the desired properties.

The aim is to obtain stable and externally resistant colloidal solutions of silver nanoparticles and to investigate the antimicrobial efficacy of synthesized nanoparticles. Silver nanoparticles (AgNPs) were obtained by crude metal synthesis by reducing and stabilizing silver nitrate in extracts from bioactive compounds.

The morphology of lyophilized AgNPs of biologically active tomato pulp used in the work was investigated by SEM methods. From **Figure 6**, the microstructures of tomato by-products AgNPs can be concluded from the irregularly shaped particles but do not form agglomerations, which will have a positive effect on antimicrobial

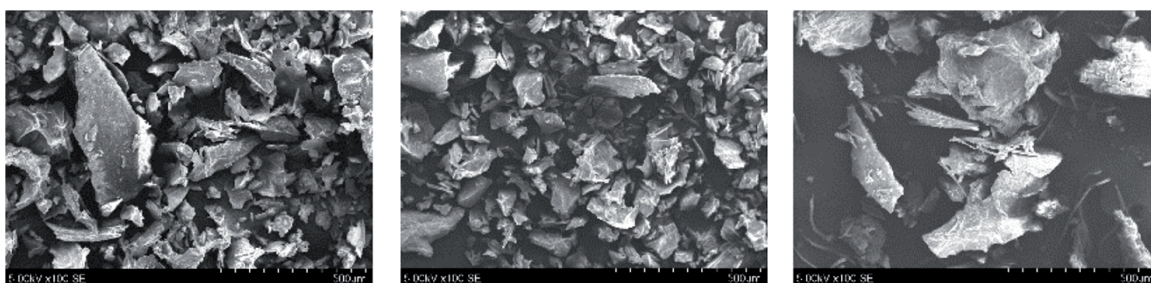


Figure 5.
SEM images of tomato by-products particles

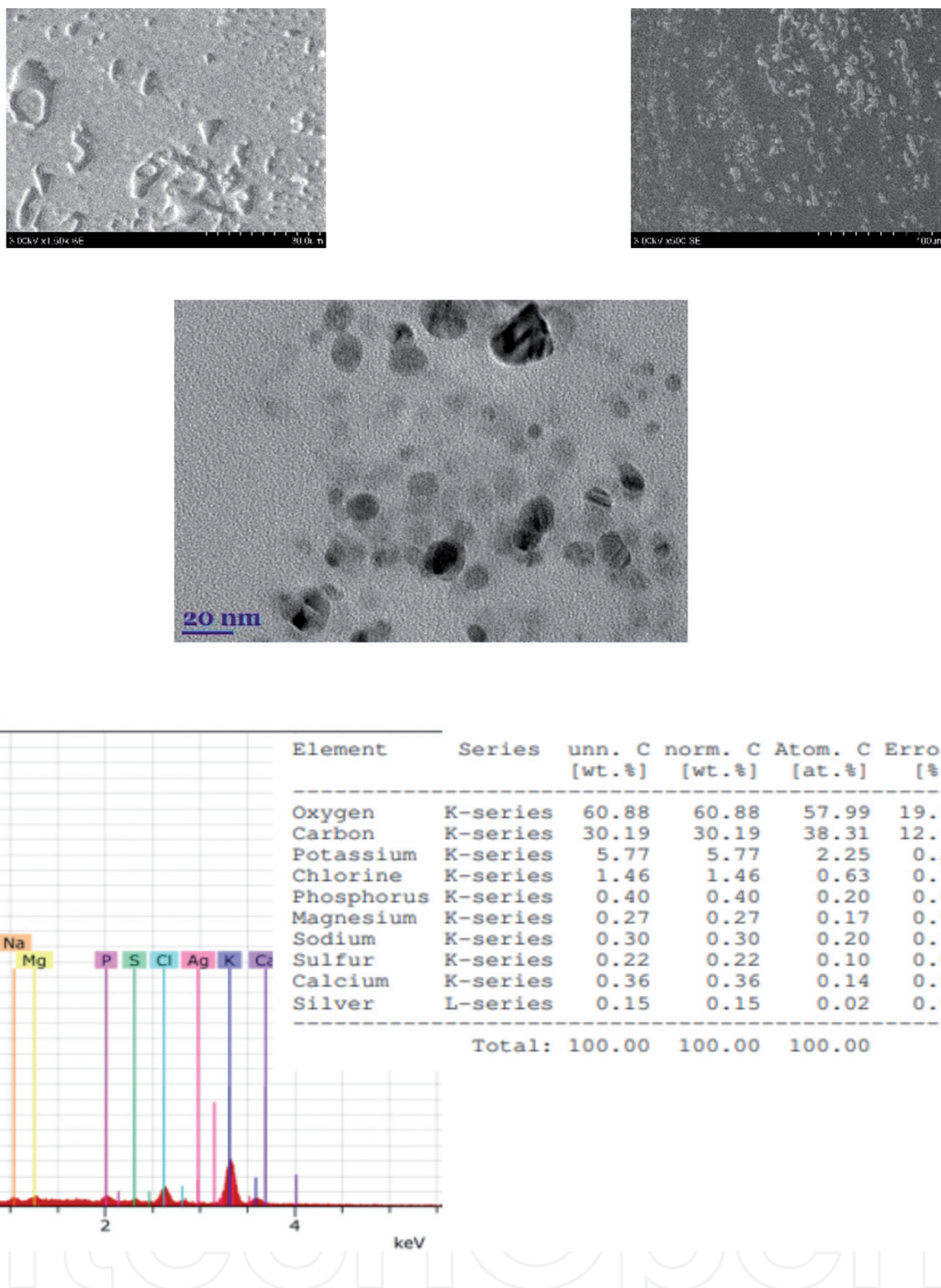


Figure 6. Tomatoes “Vilina” by-products TEM micrographs, surface EDS spectra, and elemental analysis

activity. From the photos provided by TEM, we can see a clearer morphology of the particles. The particles are spherical and do not form agglomerates (**Figure 6**).

In this case, individual agglomerations can already be observed. Scanning of metal particles at selected locations where AgNPs are suspected shows peaks in the 3.0 keV region of the EDS spectra that can be attributed to silver binding energy, and this can be detected at first and third samples. In second, sample AgNPs could not be found, but the biomatrices had antimicrobial activity. Therefore, we can say that the particles formed. With the help of TEM microscopy, we can see that the particles obtained are particles with a clear spherical shape, but in individual cases we can observe the formation of agglomerates (**Figure 7**).

The TEM images show an uneven surface with AgNPs. A high silver content in the biomatrix was identified (**Figure 8**). The particles remain irregular in shape and do not

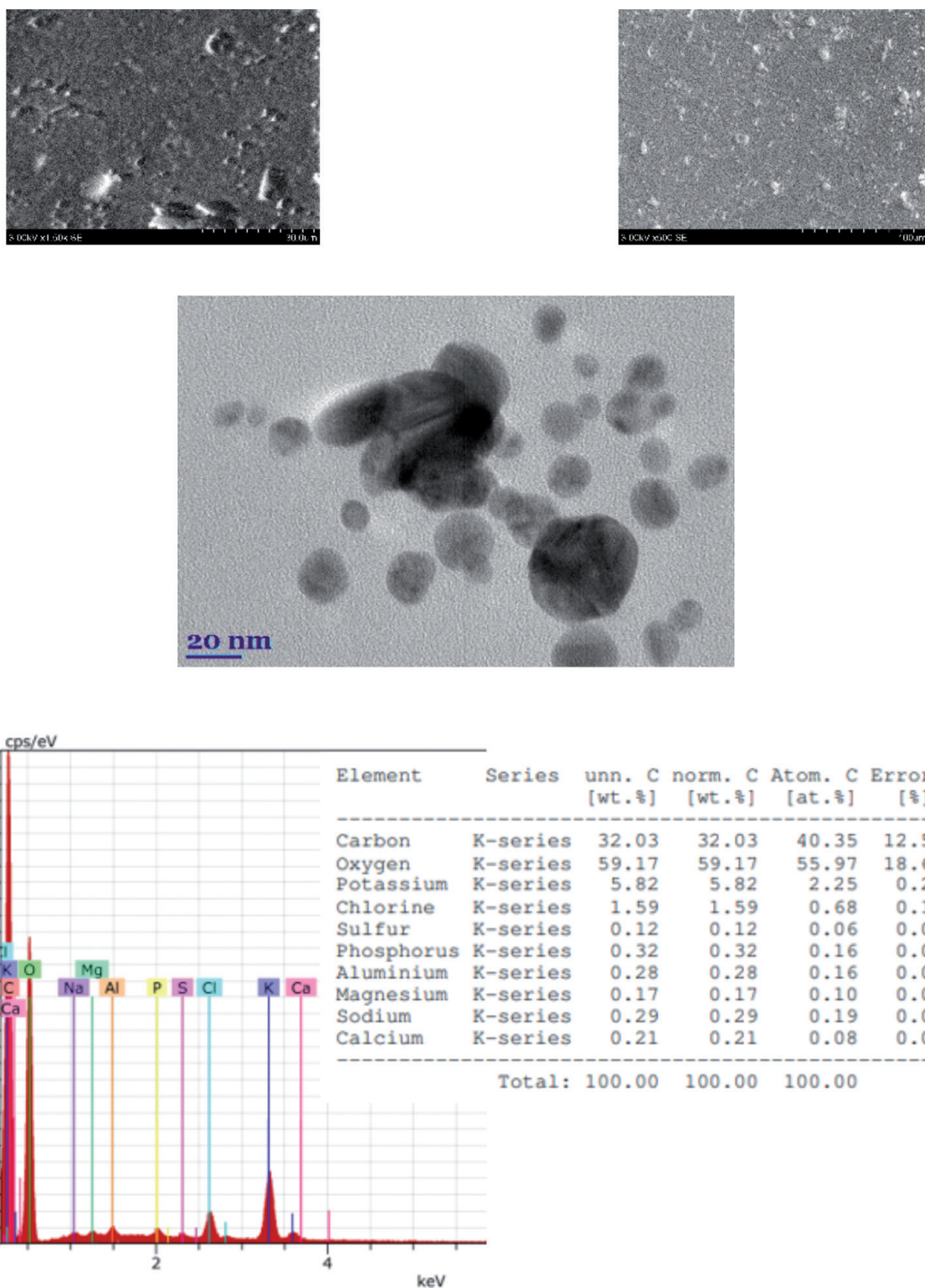


Figure 7. Tomatoes "Laukia'i" by-products TEM micrographs, surface EDS spectra, and elemental analysis

tend to form large aggregates, which is likely to have a positive effect on antimicrobial activity. From the presented photos, we can see the particle shape, size distribution, and agglomeration tendency of AgNPs. In this case, the largest particles are obtained. Also in their form the resulting spheres. The particles obtained have a relatively high polydispersity, which is likely to have a positive effect on antimicrobial activity.

The antibacterial activity of organic colloidal solutions of AgNPs was tested for both Gram-negative and Gram-positive bacterial strains and fungi. From the results

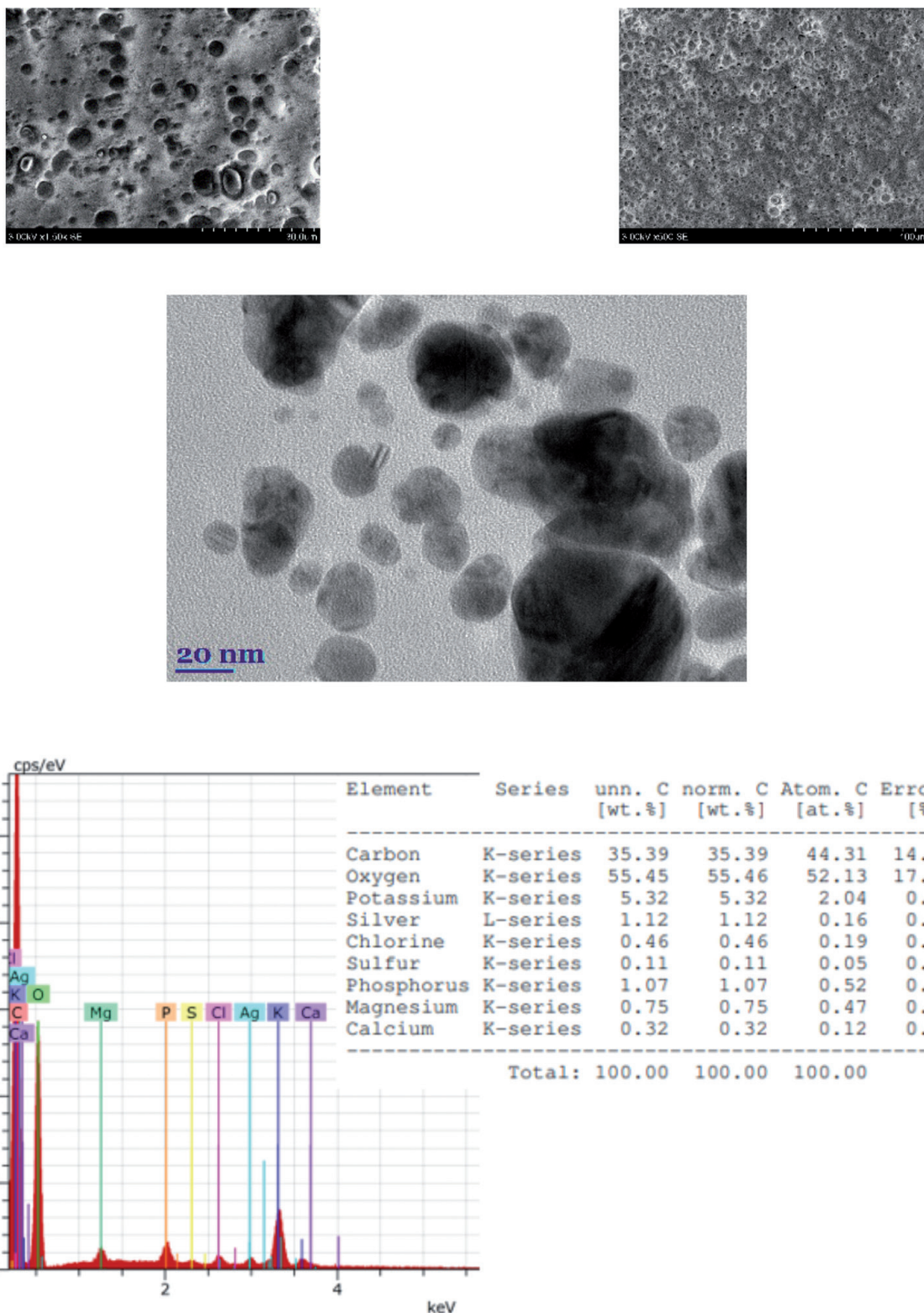


Figure 8. Tomatoes by-products mix TEM micrographs, surface EDS spectra, and elemental analysis

presented in **Table 1**, it can be concluded that silver nanoparticles in organic media actively interact with the bacterial membrane and disrupt their functions.

The results of the antifungal efficacy studies of AgNPs are presented in **Table 1**. Two different fungal cultures were selected: *Candida albicans* (*C. albicans*) and *Rhodotorula glutinis* (*R. glutinis*). In humans, *C. albicans* can cause external infections and life-threatening systemic infections, and *R. glutinis* is an opportunistic pathogen that can cause infection in

Reference (standard) cultures of microorganisms	Samples					
	1	2	3	4	5	6
<i>Staphylococcus aureus</i>	0.0 ± 0.0	10.8 ± 0.1	0.0 ± 0.0	10.10 ± 0.1	0.0 ± 0.0	12.2 ± 0.1
<i>Staphylococcus epidermidis</i>	0.0 ± 0.0	9.7 ± 0.3	0.0 ± 0.0	10.1 ± 0.3	0.0 ± 0.0	11.5 ± 0.2
<i>β- streptococcus</i>	0.0 ± 0.0	10.1 ± 0.1	0.0 ± 0.0	11.5 ± 0.1	0.0 ± 0.0	13.5 ± 0.1
<i>Escherichia coli</i>	0.0 ± 0.0	5.8 ± 0.3	0.0 ± 0.0	5.6 ± 0.2	0.0 ± 0.0	6.2 ± 0.2
<i>Klebsiella pneumoniae</i>	0.0 ± 0.0	5.3 ± 0.1	0.0 ± 0.0	4.9 ± 0.2	0.0 ± 0.0	5.5 ± 0.4
<i>Pseudomonas aeruginosa</i>	0.0 ± 0.0	4.2 ± 0.1	0.0 ± 0.0	3.5 ± 0.3	0.0 ± 0.0	4.1 ± 0.2
<i>Proteus vulgaris</i>	0.0 ± 0.0	6.8 ± 0.2	0.0 ± 0.0	6.5 ± 0.1	0.0 ± 0.0	7.1 ± 0.4
<i>Bacillus cereus</i>	0.0 ± 0.0	8.7 ± 0.2	0.0 ± 0.0	9.7 ± 0.1	0.0 ± 0.0	6.9 ± 0.2
<i>Enterococcus faecalis</i>	0.0 ± 0.0	7.6 ± 0.3	0.0 ± 0.0	8.2 ± 0.5	0.0 ± 0.0	5.4 ± 0.1
<i>Candida albicans</i>	0.0 ± 0.0	5.3 ± 0.2	0.0 ± 0.0	7.4 ± 0.4	0.0 ± 0.0	6.4 ± 0.3
<i>Rhodotorula glutinis</i>	0.0 ± 0.0	4.4 ± 0.1	0.0 ± 0.0	6.3 ± 0.2	0.0 ± 0.0	5.7 ± 0.2

Table 1.
Antimicrobial activity of the greenly synthesized AgNPs.

a weakened immune system. From the results presented in the table, it can be concluded that AgNPs obtained using different Russian tomatoes with different syntheses inhibited the growth of *C. albicans* and *R. glutinis* colonies. Meanwhile, extracts without particles did not show this effect.

It is clear that metal nanoparticles are promising as antimicrobial agents and therapeutic agents due to their biological, physical, and chemical properties. They can solve many problems in the field of nanomedicine. However, there is a lack of knowledge about the long-term effects of nanoparticles on human health and the environment. Nanoparticles are stable and can accumulate in the environment, and they have a tendency to agglomerate and can therefore change their dimensions. Toxicity studies of nanoparticles have shown that metal nanoparticles can act on the organ, tissue, cell, muscle, and protein levels. Nanoparticles are extremely small in size and can easily spread through air or water and adversely affect the skin, lungs, and brain (especially nanoparticles with dimensions ≤ 10 nm). Therefore, the search for other substances with antimicrobial activity, such as the use of plant-derived substances to obtain antimicrobial compounds, is intensifying.

5. Conclusions

Green nanoparticles obtained by green synthesis methods, which have a wide range of antibacterial properties against both Gram-negative and Gram-positive bacterial strains and fungi, expand their applications in orthopedics, biomedicine, and medicine, as well as in other industries. Recently, the range of substances resistant to microbial colonization and multiplication of pathogenic microorganisms are increasing due to the increasing use of extracts of medicinal plants and plant by-products, which are strong antioxidants with anticancer, antibacterial, anti-inflammatory, antiallergic, antiviral, hepatoprotective effects. One of the most important antioxidants accumulated in plants is phenolic compounds, the

mechanism of action of which is related to their ability to neutralize free radicals, protect against diseases caused by oxidative stresses, and reduce various forms of reactive oxygen species. It can be assumed that the modification of green nanoparticles with multifunctional hybrid particles can increase and expand their scope. Such antimicrobial and functional biomatrices are obtained using secondary by-products and Ag.

Stable colloidal solutions of AgNPs with high antibacterial activity in organic media have been obtained, which completely inhibit various bacterial cultures.

Acknowledgements

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Conflict of interest

The authors declare no conflict of interest.

Appendices and nomenclature

silver nanoparticles	AgNPs
<i>Bacillus cereus</i>	<i>B. cerues</i>
<i>Candida albicans</i>	<i>C. albicans</i>
<i>E. coli</i>	<i>E. coli</i>
<i>E. faecalis</i>	<i>Enterococcus faecalis</i>
<i>Klebsiella pneumonia</i>	<i>Klebsiella pneumoniae</i>
<i>Pseudomonas aeruginosa</i>	<i>P. aeruginosa</i>
<i>Proteus vulgaris</i>	<i>P. vulgaris</i>
<i>R. glutinis</i>	<i>Rhodotorula glutinis</i>
<i>S. aureus</i>	<i>S. aureus</i>
<i>S. epidermidis</i>	<i>Staphylococcus epidermidis</i>
β . <i>Streptococcus</i>	β eta – <i>streptococcus</i>
SEM	Scanning electron microscope
TEM	Transmission electron microscopy
MNPs	Metal nanoparticles

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