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Properties And Antimicrobial Activity of Bacterial Nanocellulose Composite Incorporated with Silver Nanoparticles Based on Green Synthesis

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Abstract

Green silver nanoparticles (Ag NPs) synthesized using medical plant extracts as capping and reducing agents demonstrate broad applicability in addressing the threat posed by infections. In this study, we present a sustainable, low-toxicity, and cost-effective bacterial nanocellulose composite with silver nanoparticles based on green synthesis that uses Origanum vulgare as a reducing agent due to numerous biological components. The formation of Ag NPs in composite and morphological properties was analyzed using scanning electron microscopy (SEM), energy-dispersive spectroscopy (EDS), and transmission electron microscopy (TEM) techniques. Antimicrobial activity was determined using the agar diffusion Kirby–Bauer method against Gram-positive and Gram-negative bacterial strains. The antimicrobial activity of bacterial nanocellulose composite/Ag NPs was substantially strong against all tested bacterial cultures. The structural investigation of Ag NPs by TEM presents the presence of spherical-shaped silver nanoparticles in cellulose composite. This method of obtaining antimicrobial composites could be widely used to fight against antibiotics, antibiotic-resistant antibiotic -resistant bacteria, and other Gram-positive and Gram-negative bacteria.

Keywords: Nanocellulose composite, silver nanoparticles, antimicrobial activity, origanum vulgare, biosynthesis.

INTRODUCTION

Nosocomial infection (NI) is any illness of bacterial, viral, parasitic, or fungal origin related to the patient's hospitalization, examinations, and treatment in a personal healthcare institution and work there.

HI includes all infections the patient did not have on arrival at the hospital and were not in the incubation period at the time of hospitalization. Since the incubation period of many infections is not precisely defined, infections usually occur after 48 hours. after hospitalization, they are registered as a hospital [1, 2].

The emergence of HI is determined by many factors: the patient's condition, microorganisms, and external factors. HI can be exogenous (acquired from outside) and endogenous (caused by microorganisms carried by the patient himself). It is often difficult to differentiate between them because a person who is in a hospital environment acquires microorganisms circulating in the hospital (mainly if he stays longer before surgery), becoming a carrier of them, and later (for example,

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after surgical intervention) these bacteria cause illness. HI belongs to infections not only of patients but also of staff acquired in inpatients and in other health care institutions (polyclinics, nursing homes, etc.), which are caused not only by bacteria but also by other microorganisms (viruses, etc.). They occur not only during treatment but also after leaving the medical institution (as the duration of inpatient treatment shortens, it occurs more and more often). Therefore, the synonym of HI is increasingly used today - healthcare-associated infections [3, 4].

The escalating threat posed by infections underscores the urgent need for the development of new substances with antimicrobial activity. Your work in this area is crucial, and this paper contributes to this vital research.

This paper presents a significant breakthrough in the field, detailing the synthesis of a natural bacterial nanocellulose composite incorporated with silver nanoparticles. This innovative approach, utilizing green synthesis with the aqueous extracts of Origanum vulgare, holds immense potential as an antibacterial material against both Gram-negative and Gram-positive pathogenic bacteria strains. Additionally, the material exhibits antioxidant properties, further enhancing its value in the fight against infections.

LITERATURE REVIEW AND OBJECTIVE

Antibiotic resistance develops when a bacterium changes in response to a drug that affects it. After this process, bacteria become resistant to antibiotics. Such bacteria can infect a person. Infections caused by them are more difficult to treat than those caused by drug-resistant bacteria.

Antibiotic resistance significantly increases healthcare costs, prolongs hospital stay and increases mortality. In the European Union only, antimicrobial-resistant bacteria are estimated to be the cause of approximately 25,000 deaths and expenses more than €1.5 billion. US dollars annually on health care costs [5].

Plants can produce various secondary metabolites such as: saponins, alkaloids, terpenoids, glycosides, steroids, flavonoids, coumarins, tannins and quinones and various other biologically active compounds. Medicinal plants have been selected as a main origin of biological active material since time immemorial. They can synthesize various chemical compounds that are important for the maintenance of biological functions such as: reproduction, growth, adaptation of pigmentation to adverse environmental conditions. Many of these phytochemicals also have beneficial effects at healthiness , effective in the treatment or elimination of numerous diseases.

Phenolic compounds - phenols, or polyphenols, are secondary metabolites of biochemical processes occurring in plants (metabolic pathways of pentose phosphates and phenylpropanoids), which can also be used in biosynthesis processes of metal nanoparticles. The structure of phenolic compounds is characterized by one to several aromatic rings that contain one or several functional hydroxyl groups. In plants, polyphenols are usually found conjugated with various mono- or polysaccharides, with one or more phenolic groups attached, or as functional derivatives of esters and methyl esters.

These compounds directly bind harmful O_2^-, $HO^•$, $ROO^•$ and $NO^•$ radicals found in biological systems, as well as non-radical $ONOO^-$, $^1O_2$, H_2O_2 and HCl acid.

Green biosynthesis, among other synthetic methods, is characterized by clear in working and process conditions, as it uses only plant-derived metabolites and metal salts [6]. Because of the chemical, electrical and antimicrobial properties, metal particles are extensively used in various industrial fields such as pharmaceuticals, medicine, health care and others [7, 8].

Bacterial nanocellulose (BNC) is a linear natural homopolymer. This material is synthesized by natural biological methods by Acetobacter culture, which is equivalent to simple growth methods. By

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applying these synthesis methods, extremely large quantities of such biopolymer can be obtained economically [9]. By applying natural growth processes and growth medium, we can obtain a pure biopolymer with targeted properties. And also, the material can be modified during growth, which depends on the physical and chemical properties. These properties are greatly influenced by: temperature, pressure, light, pH of the growth medium, enzymes. such bacterial cellulose is characterized by fibril-shaped particles that form the main matrix. Due to these properties, it is mechanically strong, has excellent tensile strength, deformation and elongation properties during stretching. In addition, it is elastic and hydrophilic, which leads to great water absorption capabilities. However, if this substance is to be used more widely in the fields of medicine, pharmacy or health care, it is necessary to modify it by giving it antimicrobial activity [10].

Materials and Methods

Green silver nanoparticles synthesis was performed. Silver nitrate (>99,5%, Fluka, Germany) were counterbalanced of 0.03 mg, after that dissolved in 3 ml pure water. Then, 30 ml of medicine plant - Origanum vulgare aqueous extract were taken and mixed with silver solution. Prepared AgNPs were stored for 24h at room temperature about + 16°C.

Determination of antimicrobial activity by diffusion into agar (Kirby–Bauer) method. In this method, bacteria are inoculated into a special Mueller Hinton agar medium. The antimicrobial effect on bacterial cultures was evaluated after 24 h of cultivation by the size of the clear zones formed around the samples, the radius expressed in millimeters. Disks isolated from the test sample are placed in a Petri dish, in which a grown culture of the test organism (bacteria) has already been spread. During incubation, the antibacterial agent begins to diffuse from the disc and inhibit bacterial growth. The diameter of each zone is compared with the standard values for a given material. The test organism may be susceptible, moderately susceptible, or resistant to the antibacterial agents tested.

Microbiological activity were investigated based on Gram-positive/negative bacterial strains:

- *Gram-positive bacteria:* Staphylococcus aureus (S. aureus); Beta hemolytic streptococcus (ß-streptococcus);
- *Gram-negative bacteria*: Klebsiella pneumoniae (K. pneumoniae), Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa) [4, 10].

Scanning electron microscopy (SEM). The surface morphology and chemical analysis of the investigated materials and their composites were investigated by SEM "FEi Quanta 200 FEG": resolution - 1.2 nm, accelerating voltage - 20 kV (FEI, United States of America). The EDS system consists of a Bruker XFlash® 4030 (FEI, USA) X-ray energy dispersive detector, signal processor, controller, and ESPRT data analysis software. The spectrometer allows quantitative and qualitative assessment of the sample's chemical composition by detecting chemical elements in the sample area and determining the distribution map of individual chemical elements on the surface. Moreover, this study allows us to qualitatively assess the morphology and structural changes of the object under study. Samples were scanned at least three different times.

RESULTS AND DISCUSSION

Morphological investigation of Composite/Ag NPs

Scanning electronic microscopy (SEM) analyzed structural properties such as size, shape, distribution of particles, and elemental composition of the matrix. Figure 1 presents silver nanoparticles obtained by green synthesis methods using oregano extract. Imagine shows that most of the Ag NPs are nearly spherical, with irregular geometric shapes. The EDS spectrum and elemental analysis of the matrix confirm that Ag NPs have been formed, and their formation is also shown by the reduction of microelements, which is observed after synthesis [11]. Also, it can be detected that silver nanoparticles are distributed evenly throughout the studied area.

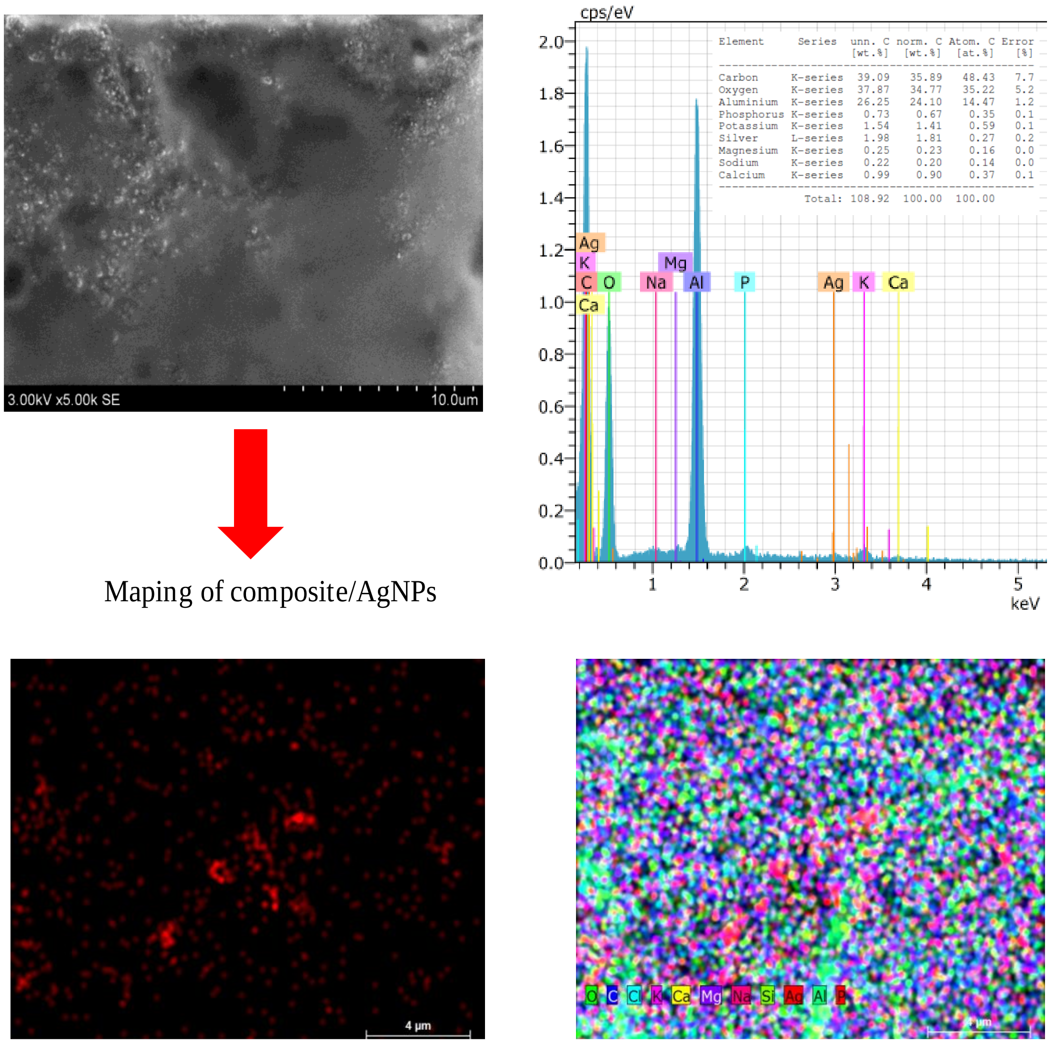

Figure 1. SEM-EDS of composite/Ag NPs synthesized based extract of O. vulgaris.

It can be detected that Ag NPs are appropriate even throughout the investigated area of the composite (Figure. 1.). This is confirmed by the obtained antimicrobial activity studies, which confirmed the excellent dispersion of green silver nanoparticles throughout the composite. The formation of O. vulgaris green Ag NPs is confirmed by the EDS spectrum. Also, from the data presented, metal particles do not tend to form agglomerates.

Biological-antibacterial activity

The biological activity of pure extracts, composites, and Ag NPs was tested against both Gram-positive and Gram-negative bacterial types. In Table 1, it can be seen that the obtained Ag NPs interact actively with the microorganism's cells and disrupt their functions very actively. No antimicrobial activity was obtained on pure extracts with E. coli, K. pneumoniae, and P. aeruginosa.

The extract of Origanum vulgaris presents antimicrobial activity, but only against *Gram-positive bacteria:* Staphylococcus aureus and ß-streptococcus. Weakly antimicrobial activity of pure extract was obtained when the size of the transparent zones was 1.0 to 1.5 mm. After synthesis of green O. vulgaris/Ag NPs in composite, stable composite/Ag NPs with strong antibacterial activity. The tested composite showed the most potent antimicrobial activity against ß-streptococcus, where the zone of inhibition was 10.2 mm. Meanwhile, the strain of Pseudomonas aeruginosa bacteria with the weakest activity was obtained - 6.3 mm.

Table 1. Antimicrobial activity of the green obtained AgNPs and composite/AgNPs

Samples	**Gram-positive and Gram-negative microorganisms**				
	Staphylococcusaureus	***ß-streptococcus***	***Escherichia Coli***	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***
Pure O. vulgare	1.00±0.2	1.5±0.1	0.0±0.0	0.0±0.0	0.0±0.0
O. vulgare/AgNPs	8.5±0.5	9.2±0.8	6.5±0.2	6.2±0.3	5.9±0.1
Composite/AgNPs	9.2±0.3	10.2±0.1	8.6±0.1	7.5±0.2	6.3±0.2

Differences in antimicrobial activity are explained based on morphological differences in Gram-positive and Gram-negative bacteria cell walls. The relatively thick wall allows even large molecules to pass through because it is porous. The wall of Gram-negative bacteria has an additional layer - an outer membrane 10-15 nm thick, which is separated from the plasma membrane by the so-called periplasmic cavity. In the outer part of the outer membrane, functional groups of molecules are densely arranged, having a negative charge under physiological conditions. Such a dense formation does not allow many chemicals to enter the cell [12].

CONCLUSIONS

The silver nanoparticles were obtained using a sustainable, green, and environmentally friendly approach based on Origanum vulgare aqua extracts as a reducing and capping agent. The size of obtained AgNPs mediated by O. vulgare ~ 27.5 nm. Hydroxycinnamic acids, phenolic compounds, flavonoids, and other phytochemicals found in Origanum vulgare extracts and metal nanoparticles obtained by green synthesis methods act synergistically, resulting in strong antimicrobial activity. Also, the antimicrobial evaluation of AgNPs and cellulose composite/AgNPs revealed strong antibacterial activity against all tested, such as Staphylococcus aureus, Beta hemolytic streptococcus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae. Our research results contribute to an eco-friendly, sustainable, and novel area of a biosynthesis approach and antimicrobial biomaterial as a perspective approach in pharmacy and medicine.

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