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Green Synthesis of Au-doped SnO_2 Nanoparticles Using Teucrium Polium Plant Extract for the Evaluation of Their Physicochemical and Antibacterial Properties

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Abstract

Background & Objective: The biological production of nanoparticles (NPs) is a technique that has garnered a lot of interest because it is inexpensive, straightforward, and friendly to the natural environment. This study explores the green synthesis of Au-doped SnO_2 NPs using Teucrium polium plant extract and evaluates their antimicrobial properties in comparison with two commonly used antibiotics.

Materials & Methods: Initially, an extract of Teucrium polium was made using water, and then it was combined with solutions of tin (II) chloride dehydrate [$SnCl_2.2H_2O$] and gold (III) chloride trihydrate [$HAuCl_4.3H_2O$]. The crystal structure was studied using X-ray diffraction (XRD). The form, structure, and grain size of the NPs were analyzed using scanning electron microscope (SEM) and transmission electron microscope (TEM). The synthesized NPs were tested for their antibacterial properties.

Results: The XRD and EDS analyses revealed that the SnO_2: Au phase formed an average crystallite size of 22 nm and an Au dopant content of around 2%. The SEM and TEM investigations demonstrated that the NPs were formed in a regular and nanoscale manner, with diameters ranging from 25 to 30 nm. MIC and MBC of SnO_2: Au NPs against the studied bacteria (S.aureus ATCC 43300, P.aeruginosa PAO1) were reported 9.6±0.13- 9.6±0.07 µg/mL and 25.9±11.5- 32.5±11.2 µg/mL.

Conclusion: Green-synthesized NPs exhibit significant antimicrobial activity against both Gram-positive and Gram-negative bacteria, even at low concentrations. The NPs effectively eliminate bacteria and prevent biofilm formation.

Keywords: Teucrium polium, Green synthesis, SnO_2: Au NPs, Structural analysis Antimicrobial

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Introduction

The bacterial infection has been an important danger to the health of people all over the world. The development of antibiotics has led to significant progress in the treatment of illnesses caused by microbes (1). However, the use of conventional antibiotics for extended periods of time and in large amounts can lead to the appearance of bacteria that have developed resistance to antibiotics, which poses a danger to public health through multiple channels, including food, water, and livestock (2, 3). A biofilm is a group of microbes that are dependent on a surface and enclosed in an environment that promotes bacterial growth. The formation of biofilms is

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possible on a wide variety of body tissues, as well as on industrial and medical device types, natural water surfaces, and virtually any other surface (4, 5). Biofilms are clinically important in several ways: they are resistant to antimicrobial compounds, become a source of infection in the body, and contain pathogenic organisms in their structure; but the problems with using antibiotics and the stability of bacteria to antibiotics have caused problems (6-8). In order to find a solution to this problem, a significant amount of work has been put into developing viable alternatives to the treatment of bacterial infections and antibiotic resistance. In the not-too-distant future, it will be very risky to rely on antibiotics. As a result, novel approaches to the management and eradication of microorganisms are receiving a lot of attention (9). Nanotechnology has shown significant promise in its ability to revolutionize antibiotic-free treatment methods, and it has achieved significant success in the implementation of antimicrobial techniques. Nanomaterials comprise a wide range of chemical compounds, which endows them with many special advantages. A useful substance with inherent antimicrobial activity can be produced by carefully controlling the physicochemical characteristics of nanoparticles, including size, surface chemistry, and form, which results in cell membrane injury. Nanomaterials can be used as transport carriers to facilitate the penetration, controlled release, and targeted absorption of bacteria from natural substances, as well as improve their stability to suppress bacteria. Alternatively, they can be used as supplements in vaccine compositions, which improves their efficacy while simultaneously lowering the required dosage (10-12). The NPs connect to the bacterial film through an electrostatic response and cause annihilation by disturbing it. Another way that antimicrobials work is by damaging the cell wall of bacteria through a reaction with the amines and carboxyl groups present in the peptidoglycan layer. Conversely, the effectiveness of metal oxide nanoparticles in killing bacteria depends on the rate at which ions are released and reactive oxygen species are produced (13, 14). NPs can generally be divided into two categories: carbon and metal. The use of metal oxide NPs in a wide range of microorganisms had antimicrobial effects that can be a good alternative to antimicrobial compounds (15). Metal oxide NPs such as aluminum NPs against Escherichia coli (16), bismuth ferrite NPs against *Staphylococcus aureus* (17), metal oxide NPs against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli* have been used (18) but their effects on microorganisms and inhibition of biofilms have not been studied. The enhancement of both the physical properties and efficacy of metal oxide nanoparticles can be achieved through the process of doping with elements that elicit alterations in their structural, electrical, and optical characteristics. This is especially accomplished by introducing materials that possess surface plasmonics and properties akin to those of noble metals. These materials are used in imaging and biomedical applications (19-21). One of the most widely used materials is gold (Au), due to its biocompatibility and high chemical stability. Modifying and doping NPs using Au metal, improves the surface of NPs and increases their efficiency as antimicrobial, anti-cancer, and anti-tumor mediators (22). SnO_2 metal oxide NPs, which is known as a semiconductor that belongs to groups I-VII of the periodic table, have a crystalline structure of tetragonal NPs in the form of n-type semiconductors and direct bandgap. These NPs have numerous uses in the areas of solar cells, batteries, diodes, gas sensors, and most significantly, conducting antimicrobial and numerous biological activities due to their special characteristics (23-25). Several techniques for producing and doping metals in SnO_2 metal oxide NPs have been described,

including chemical sol-gel, solvothermal, and co-precipitation. The co-precipitation technique is the easiest, least costly, and most efficient way for producing NPs (23). The use of plants to make NPs is a bio-environmentally friendly approach. Plants have biological properties and are stable and biocompatible. Because of the properties of substances present in their structure, they reduce the bio-metal ions, so the green synthesis by plants creates different sizes for NPs, often in the spherical shape with extensive applications in medical fields (26). In this research, SnO_2: Au NPs have been biologically synthesized, which are environmentally friendly and have low manufacturing costs. The medicinal extract of the Teucrium polium plant has been used for the synthesis of NPs as a stabilizer and reducing agent and its antibacterial properties were studied in this work. The use of NPs in inhibiting the biofilm formation of pathogenic bacteria was confirmed. Due to the importance of these bacteria in public health, NPs can be used to prevent the formation of biofilms.

Materials and Methods

Production of Teucrium polium plant extract

Production of NPs uses extracts from different parts, without extrinsic capping substances, surfactants or templates. The plant-derived extract includes phytochemicals and biomolecules, such as flavonoids, polyphenols, phenolic acids, terpenoids and alcohols, which stabilize and reduce agents for metal ions or their precursor (27). The Teucrium polium plant was collected and washed thoroughly with distilled water. The plant material was dried in a dark and cool place and ground to a fine powder, combined with 50 mL of deionized water, mixed, and heated at 60 °C for 30 min. The Whatman filter paper was used to filter the resultant solution before it was used to create SnO_2: Au NPs.

Synthesis of SnO_2: Au NPs

SnO_2: Au NPs were synthesized using the co-precipitation method. In this regard, Tin (II) chloride dehydrate [$SnCl_2.2H_2O$], and Gold (III) chloride trihydrate [$HAuCl_4.3H_2O$] were purchased from Merck and the high purity herbal distillation of Teucrium polium was used without further purification.

In this experiment, 0.75 g of tin (II) chloride and 1 mole % of the gold salt, $HAuCl_4$, were blended together in deionized water. After that, the solution was homogenized for 20 minutes at (25°C) using a magnetic agitator, and 10 mL of the Teucrium polium distillation was added to the tin solution. A brown precipitate was produced after 10 min, and it was separated using centrifugation and washing with double-distilled water. The resulting precipitate was dried in an oven for 24 h. The dried precipitate was placed in the furnace for 2 h at 600 ºC to eliminate additional material. Figure 1 illustrates the green synthesis of SnO_2: Au NPs using the co-precipitation techniques.

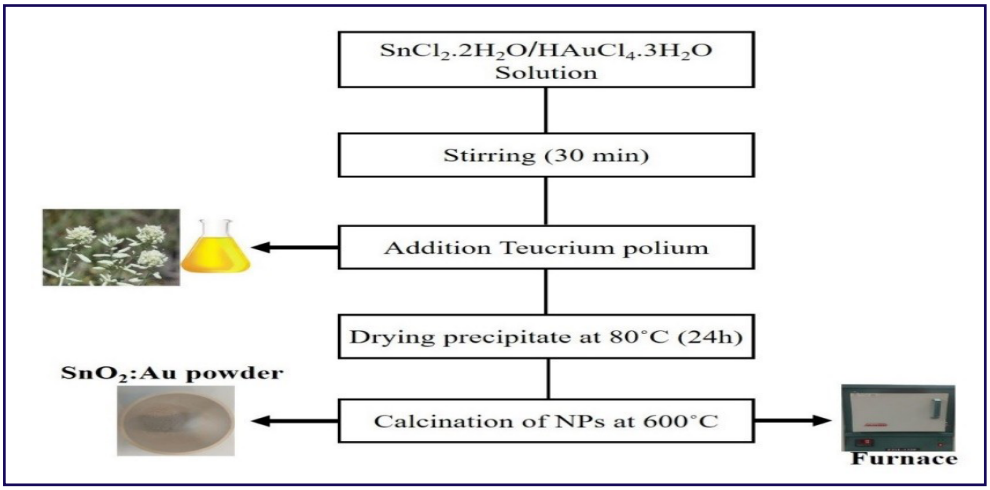

Figure 1. The diagram shows the synthesis process of SnO_2: Au NPs by the co-precipitation method

Characterization of the SnO_2: Au NPs

With the aid of X-ray diffraction (XRD, Philips), an examination of the crystal structure was conducted. A scanning electron microscope (SEM; model: TESCAN Mira 3-XMU) and the shape, structure, and grain size of the NPs were analysed using a transmission electron microscope (TEM; model: Zeiss EM900). A Fourier transform infrared spectrometer (Magna-IR550 model) was utilized to ascertain the presence or absence of functional groups.

Antibacterial activity assay

S. aureus ATCC 43300 and *P. aeruginosa* PAO1 were the two strains of bacteria that were used in this research to test the antimicrobial efficacy of the SnO_2: Au nanoparticles.

To determine the MIC, the researchers employed the broth microdilution method, following the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI). In a nutshell, sterile 96-well plates had 100 µL sterile Mueller Hinton broths poured into each well. Following that, a dilution sequence of SnO_2: Au Nanoparticles was prepared and applied to each well. In the end, each well was injected with 10 µL of a bacterium solution holding about 5×10^5 CFU. The plates could be incubated for 18-20 h at 37°C. The MIC was determined to be the minimum concentration of SnO_2: Au NPs that prevented bacterial development.

10 µL were taken from the MHA plate wells that did not have any bacterial development. Colony forming units (CFU) have all been found after a 24-hour period of culture at 37°C. MBC was found to be the lowest quantity that effectively eliminates 99.9% of the microorganisms.

The microtiter plate (MtP) technique is a qualitative experiment used to determine the possibility that SnO_2: Au NPs have an inhibiting impact on the development of biofilm After preparation in TSB, the bacterial suspension might have been brought to a concentration of 10^6 CFU/mL. The subsequent step was to inoculate sterile 96-well MtP plates with 200 µL of cell suspension. Then SnO_2: Au NPs (concentrations of 1 MIC, $\frac{1}{2}$ MIC, $\frac{1}{4}$ MIC, $\frac{1}{8}$ and MIC) were added to each well. MtP was subjected to incubation for 24 h at a temperature of 37°C. Following the incubation period, the wells were rinsed carefully three times with a volume of phosphate-buffered saline (PBS) containing 200 µL each time to remove non-adherent cells. After the air-drying process, wells were stained with 200 µL of 0.1% crystal violet for 15 minutes. 200 µL of 33% glacial acetic acid was used to resolubilize the crystal violet. Afterwards, a microplate scanner was used to measure the optical density (OD) at 570 nm.

Results

Analyzing the Morphology, Composition, and Structure

SnO_2: Au NPs XRD patterns are shown in Figure 2 (a). XRD analysis of Au doped SnO_2 can reveal information about the crystal structure, lattice parameters, and crystal orientation of the material. It can also be used to identify the presence of impurities, as well as the degree of crystallinity and the size and shape of crystallites. The illustration demonstrates how the heat treatment causes the NPs to transition into a crystal phase. Therefore, XRD peaks visible at 2θ°= 26.7°, 34.5°, 38.1°, 52.2°, 54.9°, 58.3°, 62.2°, 65.1°, 71.4°and 79.0° are assigned to SnO_2(110), SnO_2(101), SnO_2(200), SnO_2(211), SnO_2(200), SnO_2(002), SnO_2(310), SnO_2(122), SnO_2(202) and SnO_2(321) planes (in accordance with the JCPDS card no. 0-002-1340), This means that the SnO_2: Au phase with a Tetragonal structure has been formed. The (101) plane lattice constant, which is the same thing, is found to be a=3.73 Å. Furthermore, the typical size of the crystallites is around 18 nm, and the XRD pattern shows that there is no secondary phase (28, 29). It is likely that the exceedingly low concentration of element Au found in the SnO_2 combination is to blame for the paucity of peaks pertaining to that element. On the reverse hand, the EDS spectrum of the SnO_2: Au NPs is displayed in Figure 2 (b). It has been established that the elements Sn, O, and Au are present and their percentages of atomic make-up add up to 69.90%,

28.55 %, and 1.55%, respectively. It has been determined that the concentration of the Au dopant is less than 2%, which is an amount that is in agreement with the stoichiometric ratio.

SnO_2:Au NPs are depicted in an upper SEM image in Figure 3 (a). The NPs are seen to be aggregated, creating a surface that is fairly continuous and homogeneous. Teucrium polium, which was used in the biochemical synthesis method, seems to have the ability to function as a surfactant, leading to the creation of tiny, homogeneous SnO_2:Au NPs. TEM research was used to thoroughly examine the particle sizes of the calcined NPs.

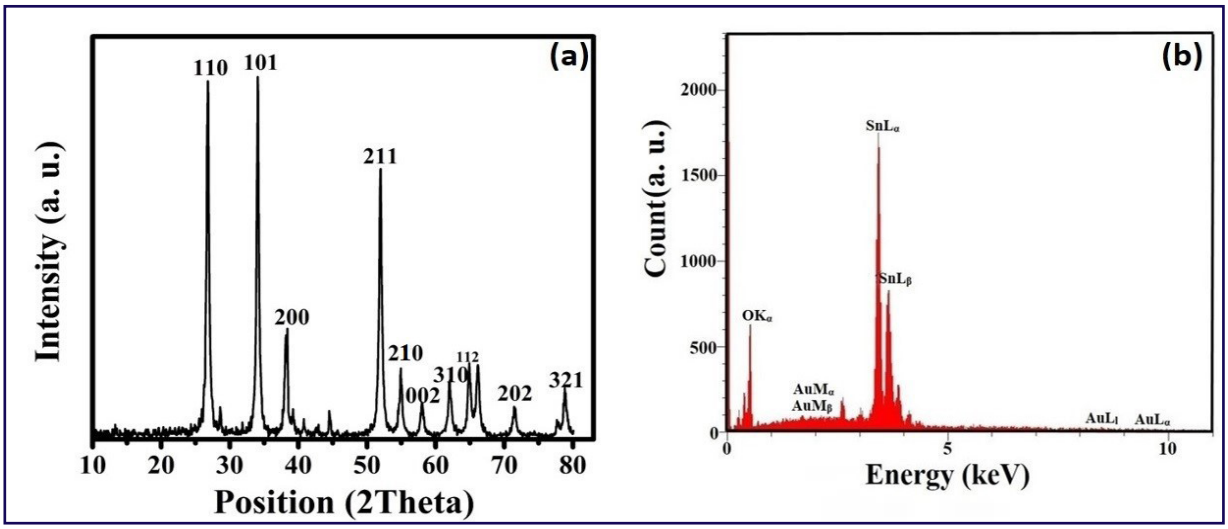

Figure 2. (a) The XRD pattern of SnO_2: Au NPs. (b) EDX image of SnO_2: Au NPs

The TEM picture of the NPs is shown in Figure 3 (b), and the size distribution histogram is shown in the inset of the same figure. Due to their small size, the majority of the particles have acquired a spherical shape and are agglomerated. Small particles have a greater tendency to agglomerate due to their intense interaction with one another as a result of attraction forces (28). The NPs are extremely small and appear to be evenly distributed throughout the sample. Additionally, the diameter of sphere NPs is typically around 22 nm, with a range of 20-25 nm.

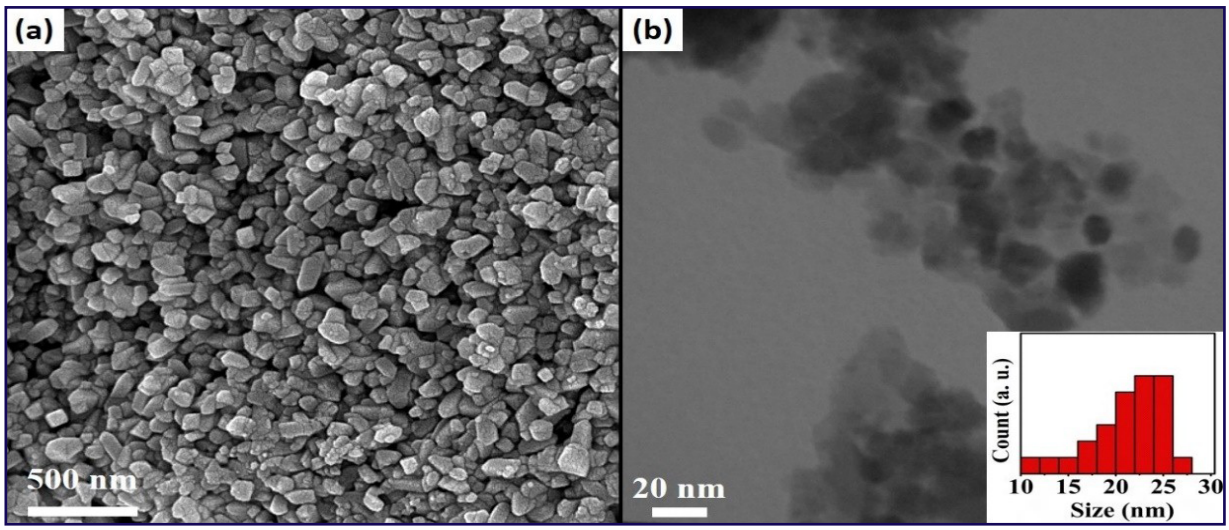

Figure 3. (a) SEM images of SnO_2: Au NPs, and (b) TEM images of SnO_2: Au NPs. The inset in part (b) of the image displays a histogram indicating the size distribution of the NPs

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FTIR Spectroscopy

When an NPs sample is exposed to infrared light, it results in a shift in the energy associated with bond vibrations, and transmittance is determined as a function of wavenumber for chemical reactions in FT-IR spectroscopy. The fingerprint area, which includes the wavenumber range from 400 to 1500 cm^{-1}, reveals the presence of individual molecule oscillations in the NPs sample. For this, we have FTIR bands of SnO_2: Au NPs. The results of the study in Figure 4 show the 400–4000 cm^{-1} wave number region. Bands at 636, 1152, 1627, and 3386 cm^{-1} allocated into O-Sn-O bonds, Sn-O bonds, O-H bonds, and O-H bonds, respectively (30).

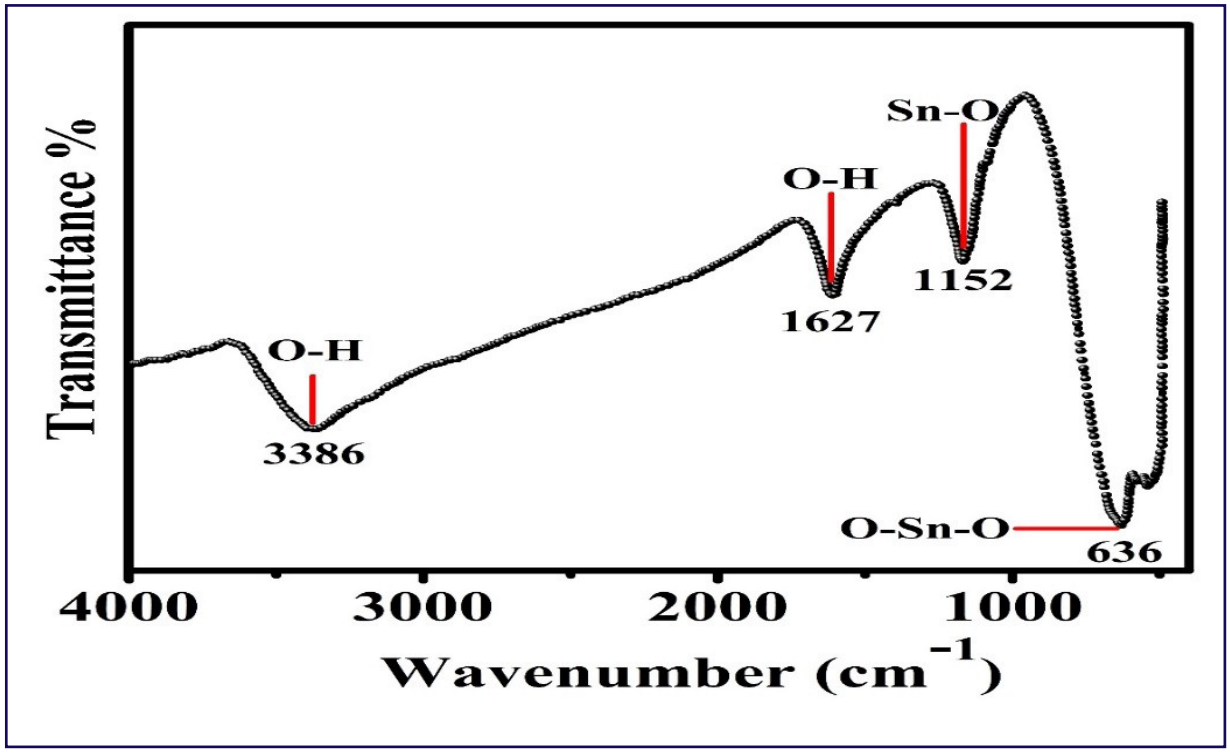

Figure 4. FT-IR spectra of SnO_2: Au NPs

Discussion

SnO_2 and Au are both materials that have been investigated for their antimicrobial properties. Studies have demonstrated that SnO_2 has antimicrobial properties against various microorganisms, such as bacteria, fungi, and viruses. The antimicrobial activity of SnO_2 is attributed to its capacity to produce reactive oxygen species (ROS) upon exposure to light, which can damage the cell membranes and DNA of microorganisms, leading to their inactivation. Studies have also revealed that Au NPs possess antimicrobial properties against different types of microorganisms, such as bacteria, fungi, and viruses.

The antimicrobial activity of Au NPs is thought to be due to their ability to interact with cell membranes and disrupt their integrity, leading to cell death (31-34). When SnO_2 and Au are combined, the resulting material, SnO_2: Au, has been shown to exhibit enhanced antimicrobial activity compared to either material alone. The increased antimicrobial activity is believed to result from a synergistic interaction between the two substances, in which the Au NPs serve as a catalyst for the production of ROS by SnO_2, resulting in increased activity. The antimicrobial efficacy of SnO_2: Au NPs was surveyed by calculating the MIC and MBC using broth microdilution; The findings are presented in Table 1. The results reveal the considerable antibacterial

activity of SnO_2: Au NPs against *S. aureus* and *P.aeruginosa*. In general, three mechanisms can be proposed for its increased chemical and biological activity: cell membrane disruption, free radical formation, and high ratio of surface area to volume. Overall, the combination of SnO_2 and Au shows promise as a material for the development of antimicrobial coatings, surfaces, and other applications where the control of microbial growth is important. However, additional investigation is necessary to obtain a complete understanding of the matter the mechanisms of antimicrobial activity of SnO_2: Au and to optimize its properties for specific applications.

Table 1. The MIC and MBC values of SnO_2: Au NPs were determined for standard strains of *S. aureus* ATCC 43300 and *P. aeruginosa* PAO1

Microorganism	NPs: SnO_2:Au	
	MIC(µg/mL) (Mean ± SD)	MBC(µg/mL) (Mean ± SD)
S.aureus ATCC 43300	9.6±0.13	25.9±11.5
P.aeruginosa PAO1	9.6±0.07	32.5±11.2

The presence of SnO_2: Au NPs inhibits the formation of biofilm:

The existence of SnO_2: Au NPs can prevent the development of biofilms. SnO_2: Au NPs are nanoparticles composed of SnO_2 and Au and are known for their antimicrobial and antibiofilm properties. The exact mechanism by which SnO_2: Au NPs inhibits biofilm formation is not fully understood, but it is believed to be related to their ability to disrupt bacterial cell membranes and interfere with the signaling pathways that bacteria use to communicate with each other and form biofilms. Additionally, the Au component of the nanoparticles has been shown to have a synergistic effect with the SnO_2 component in inhibiting biofilm formation. Overall, the presence of SnO_2: Au NPs has the potential to be a promising approach to preventing biofilm formation and reducing the associated health and economic costs.

In order to determine how much SnO_2: Au Nanoparticles could inhibit the growth of biofilms caused by MRSA and PAO1, the researchers used the microtiter plate technique. Chart. 1 shows the effectiveness of different concentrations of SnO_2: Au NPs in preventing the formation of biofilms. The greatest reduction in biofilm formation is observed in environments with higher concentrations of SnO_2: Au NPs (MIC). As a result, SnO_2: Au NPs at MIC concentration inhibited the biofilm formation by MRSA and PAO1 with a dissuasion rate of 80% and 79% respectively. The uniqueness of the present research lies in the fact that there has been no previous report regarding the antimicrobial activity of SnO_2: Au Nanoparticles and the prevention of biofilm development. Although the mechanism of SnO_2: Au NPs in preventing biofilm formation is not fully known, there are a few different suppositions floating around regarding their mode of operation. Some studies also indicate that SnO_2: Au NPs might attach to the biofilm surface and thus destroy the biofilm.

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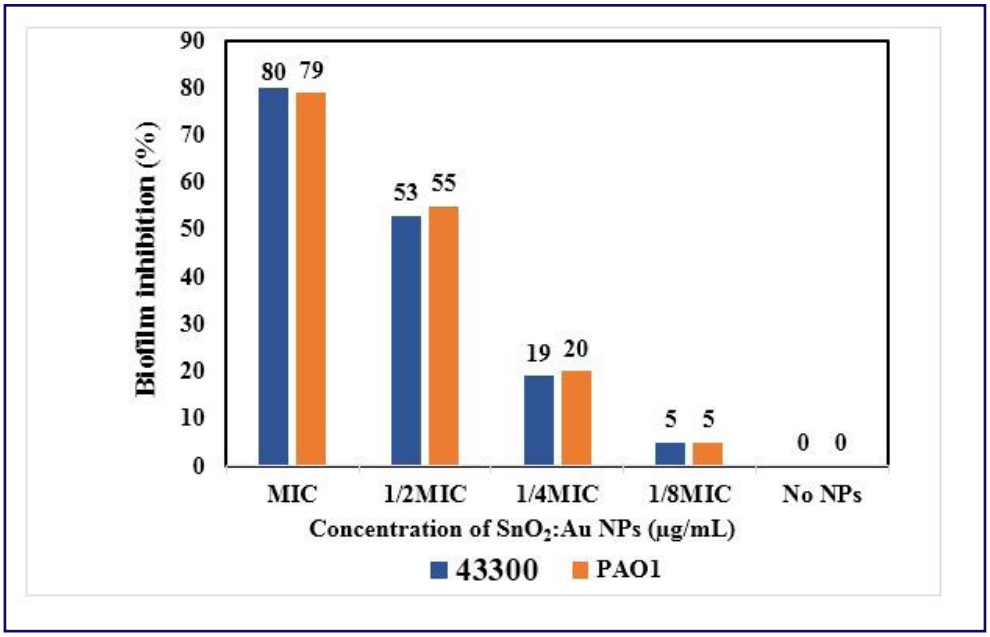

Chart 1. SnO_2: Au NPs at different concentrations on the inhibition of biofilm formation in standard strains of *S. aureus* ATCC 43300 and *P. aeruginosa* PAO1

Discussion

Co-precipitation techniques were used to synthesize SnO_2: Au NPs, which were then calcined at 600°C. The SnO_2:Au phase formed, as shown by the XRD and EDS studies, by an average size of the crystallite of 22 nm and an Au dopant content of about 2%. The results of the SEM and TEM studies showed that spherical NPs with diameters varying from 20 to 25 nm were formed in a reasonably regular and nanoscale manner. However, the FT-IR studies for SnO_2: Au NPs revealed the formation of Sn-O bonds. High minimum-maximum MIC and MBC values were found, indicating that the synthesized NPs have good bactericidal action for the first time. Increasing the NP concentration above the 1/8 MIC level resulted in a significant increase in biofilm disintegration. In conclusion, the study has demonstrated that the synthesized Au doped SnO_2 NPs have excellent antibacterial action targeting both gram-positive and gram-negative bacteria. Based on the findings, it can be inferred that these nanoparticles can be potentially used as an effective antibacterial agent in various biomedical and environmental applications. Additional investigations are warranted to examine how these nanoparticles work to combat bacteria and the extent to which they can be potentially harmful.

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Conflict of Intrests

The authors certify that there is no actual or potential conflict of interest in relation to this article. All data generated or analyzed during this study are included in this published article.

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