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Synthesis of Ag/CeO_2 Nanocomposites with Noni Leaf Extract and Its Potential Antibacterial Properties

Laellia Denada[1*], Poedji Loekitowati Hariani[1], Nabila Aprianti[2]

[1] Master's Program in Chemistry, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Ogan Ilir, Indonesia, 30662.
[2] Research Center for Energy Conversion and Conservation, National Research and Innovation Agency (BRIN), B.J. Habibie Science and Technology Park, South Tangerang, Indonesia, 15313.

[*]*Corresponding Author:* laelia12356@gmail.com

Abstract

This study aims to perform green synthesis of Ag/CeO_2 nanocomposites using noni leaf extract and to test their potential antibacterial activity. The synthesized nanocomposites were characterized using X-ray Diffraction (XRD) to determine crystallite size, Scanning Electron Microscope-Energy Dispersive Spectroscopy (SEM-EDX) to observe morphology and elemental composition, and Fourier Transform Infrared Spectroscopy (FTIR) to identify functional groups. The XRD pattern showed the formation of typical Ag/CeO_2 peaks with an average crystallite size of 15.28 nm. SEM analysis revealed morphology in the form of aggregates with varying sizes. EDX results confirmed the presence of Ag, Ce, and O elements. FTIR analysis showed the presence of typical Ce–O group absorption bands, which supports the successful synthesis of Ag/CeO_2. The synthesized nanocomposites were then tested for their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* bacteria. Antibacterial activity tests showed the formation of an 8 mm zone of inhibition (ZOI) at a concentration of 5000 ppm, demonstrating the potential of the synthesized green Ag/CeO_2 as an antibacterial agent. This study confirms that the use of noni leaf extract as a reducing and stabilizing agent is an environmentally friendly approach and has potential for environmental applications.

Keywords: Green synthesis, Ag/CeO_2; noni leaf extract; antibacterial

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Abstrak (Indonesian)

Penelitian ini bertujuan untuk melakukan sintesis hijau nanokomposit Ag/CeO_2 menggunakan ekstrak daun mengkudu dan menguji potensi aktivitas antibakterinya. Nanokomposit yang disintesis dikarakterisasi menggunakan metode XRD untuk menentukan ukuran kristalit, Spektroskopi SEM-EDX untuk mengamati morfologi dan komposisi unsur, dan Spektroskopi Infra Merah (FTIR) untuk mengidentifikasi gugus fungsi. Pola difraksi XRD menunjukkan pembentukan puncak Ag/CeO_2 yang khas dengan ukuran kristalit rata-rata 15,28 nm. Analisis SEM mengungkapkan morfologi berupa agregat dengan ukuran yang bervariasi. Hasil EDX mengonfirmasi adanya unsur Ag, Ce, dan O. Analisis FTIR menunjukkan keberadaan pita serapan gugus Ce–O yang khas, yang mendukung keberhasilan sintesis $Ag\text{-}CeO_2$. Nanokomposit yang disintesis kemudian diuji aktivitas antibakterinya terhadap bakteri *Escherichia coli* dan *Staphylococcus aureus*. Uji aktivitas antibakteri menunjukkan pembentukan zona hambat (ZOI) berukuran 8 mm pada konsentrasi 5000 ppm, yang menunjukkan potensi Ag/CeO_2 hasil sintesis sebagai agen antibakteri. Studi ini menegaskan bahwa penggunaan ekstrak daun mengkudu sebagai agen pereduksi dan penstabil merupakan pendekatan yang ramah lingkungan dan berpotensi untuk aplikasi lingkungan.

Kata Kunci: Sintesis hijau; Ag/CeO_2; ekstrak daun mengkudu; antibakteri

INTRODUCTION

Cerium oxide (CeO_2) is a rare earth oxide that has been widely studied due to its interesting chemical and biological properties, such as high stability, biocompatibility, the ability to form oxygen, and a redox potential that supports antibacterial activity [1]. However, the antibacterial efficacy of pure CeO_2 is still not optimal, so material modification efforts are needed to improve its performance [2]. This is important considering the increasing threat of bacterial resistance to conventional antibiotics, making the development of alternative nanomaterial-based antibacterial agents increasingly urgent [3].

One widely reported modification strategy is noble metal doping [4]. Silver (Ag) is known to have strong antibacterial activity, primarily through the release of Ag^+ ions, which can attach to cell walls, damage membranes, and disrupt bacterial metabolism, leading to cell death [5]. The combination of Ag and CeO_2 has been shown to produce good synergy, where the presence of Ag not only increases antibacterial effectiveness but also improves the surface properties of the material, thereby expanding interactions with bacterial cells [6]. Thus, Ag doping is a relevant approach to enhance the antibacterial potential of CeO_2.

In addition to material modification, synthetic approaches are also an important factor in the development of antibacterial nanocomposites. Conventional methods generally involve the use of synthetic chemicals that are potentially hazardous and environmentally unfriendly [7]. Therefore, green synthesis using plant extracts is increasingly being chosen because it utilizes natural biomolecules such as flavonoids, alkaloids, tannins, and saponins as reducing agents and nanoparticle stabilizers [8]. Noni leaf extract (*Morinda citrifolia* L.) is one promising candidate, because in addition to being rich in bioactive compounds, it also has innate antibacterial activity against both gram positive and gram negative bacteria [9]. To support this approach, phytochemical tests were also conducted on noni leaf extract to identify the presence of active compounds that play a role in the reduction and stabilization process of nanoparticles.

This research was conducted to synthesize Ag/CeO_2 nanocomposites through a green synthesis approach using noni leaf extract. This research is expected to produce environmentally friendly antibacterial materials and offer potential environmental applications as an alternative to addressing bacterial resistance issues.

MATERIALS AND METHODS

Materials

The materials used include $AgNO_3$ from Merck PA, 25% ammonia (NH_3), iron (III) chloride ($FeCl_3$) from Merck PA, cerium (III) nitrate hexahydrate ($Ce(NO_3)_3 \cdot 6H_2O$) from Merck PA, dimethyl Sulfoxide (DMSO) from Merck PA, ethanol (C_2H_6O) 96% from Merck PA, filter paper, disc paper, chloroform ($CHCl_3$) from Merck PA, sodium hydroxide (NaOH) from Merck PA, nutrient agar from Merck PA, Meyer reagent from Merck PA, Dragendorff reagent from Merck PA, noni leaves from Indralaya.

Methods

Noni leaf extraction

In the extraction process, the washed noni leaves were then dried and ground. 25 g of noni leaf powder was taken, and 250 mL of 96% ethanol was added, in a ratio of 1:10 (solute: solvent) [10]. After that, the solution was left for 24 hours at room temperature in a room without light. Next, the solution was filtered with Whatman No. 1 filter paper to remove any remaining impurities. The noni leaf extract filtrate was obtained and preserved in the refrigerator for further use.

Phytochemical test of noni leaf extract

Flavonoid testing was performed using the Wilstater method. 1 mL of extract was placed in a test tube, 0.5 g of Mg and 1 mL of concentrated HCl were added, and then shaken. The formation of an orange precipitate indicated the presence of flavonoids [11].

A total of 5 mL of extract was placed in a test tube. 1 mL of chloroform was heated and cooled, then added to the test tube, and a drop of Liberman-Burchard reagent was added. A purple precipitate indicated the presence of triterpenoids, and a green precipitate indicated the presence of steroids.

A total of 1 mL of extract was heated in a test tube containing 10 mL of distilled water. A few drops of 1% ferric chloride ($FeCl_3$) were added to the extract. A brownish-green or blue coloration indicated the presence of tannins.

A total of 1 mL of extract was placed in a test tube, heated, and mixed with one drop of Mayer's reagent and one drop of Dragendroff's reagent. The formation of a white precipitate in Meyer's reagent and an orange precipitate in Dragendroff's reagent indicated a positive result for alkaloids.

A total of 1 mL of extract was heated with 10 mL of distilled water, cooled, and then shaken vigorously for 10 seconds. A positive result was indicated by the formation of foam 1-10 cm high [10].

Green synthesis of Ag-CeO$_2$ with noni leaf extract

The CeO$_2$/Ag catalyst was synthesized by the coprecipitation method with a yield of 2.4 g. 1 g of AgNO$_3$ was dissolved in 100 mL of distilled water, and 4.5 g of Ce(NO$_3$)$_3$·6H$_2$O was dissolved in 50 mL of distilled water. Then, 50 mL of noni leaf extract was added to the mixture of AgNO$_3$ and Ce(NO$_3$)$_3$·6H$_2$O, stirred at 350 rpm at room temperature for 30 minutes. 25% ammonia solution was added dropwise until pH 10 was reached. The coprecipitate obtained was separated by centrifugation at 5000 rpm for 10 minutes, then washed with distilled water until the pH was neutral and calcined at 500 °C for 5 hours.

Characterization Nanocomposite

Ag/CeO$_2$ nanocomposites were characterized using the XRD SmartLab Rigaku. Ag-CeO$_2$ nanocomposites synthesized using noni leaf extract were solidified in metal wells and analyzed using FT-IR spectroscopy. The morphology and elemental composition of the Ag-CeO$_2$ nanocomposites were determined using a Scanning Electron Microscope-Energy Dispersion X-ray Spectroscopy (SEM-EDS) JOEL JSM-IT200.

Antibacterial activity test

Antibacterial activity test was conducted using the Kirby-Bauer disc diffusion method with a 5 mm diameter paper disc. The test bacteria used were *Staphylococcus aureus* and *Escherichia coli*. The nanocomposite was dispersed in DMSO solvent then vortexed for 5 minutes to obtain a homogeneous suspension. Each paper disc was dripped with 20 µL of nanocomposite solution with a concentration variation of 1000–5000 mg/L. As a comparison, a positive control (Amoxicillin) and a negative control (DMSO) were used. The disc containing the solution was placed on a solid medium that had been inoculated with the test bacteria, then incubated at 37 °C for 24 hours. After incubation, antibacterial activity was observed based on the formation of a clear zone around the disc (inhibition zone). The test was carried out three times.

Inhibition zone = inhibition zone diameter – well diameter.

RESULT AND DISCUSSION

Phytochemical Test Results of Noni Leaf Extract

Qualitative analysis was conducted to identify the secondary metabolites contained in noni leaf extract. These secondary metabolites are known to play a crucial role in stabilizing the resulting nanomaterials and contributing to their antibacterial activity. The identification process was carried out using various reagents, where the presence of the compounds is indicated by the appearance of characteristic colour changes in the samples, as detailed in **Table** 1. and **Figure** 1.

Table 1. Phytochemical test results of noni leaf extract

No.	Compound	Result
1	Flavonoid	Positive (+) There is a colour change to greenish yellow
2	Steroid	Steroid Positive (+) There is a green precipitate
3	Tannin	Positive (+) There is a colour change to brownish green
4	Saponin	Positive (+) There is foam as high as ± 3cm
5	Alkaloid	Positive (+) in Dragendorff reagent There is an orange precipitate
		Negative (-) in Mayer's Reagent No white precipitate

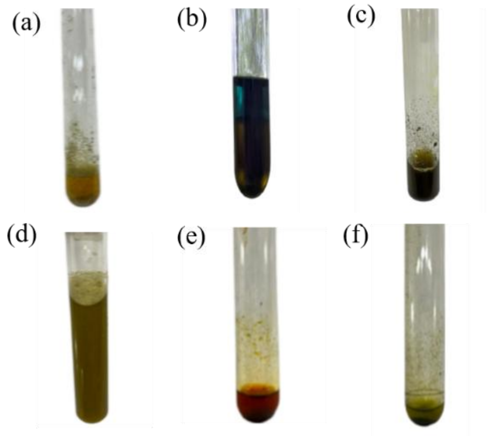

Figure 1. Phytochemical test results a) flavonoid, b) steroid, c) tannin, d) saponin, e) alkaloid in Dragendorff reagent, f) alkaloid in Mayer reagent

Qualitative analysis of noni leaf extract revealed the presence of several secondary metabolites, including flavonoids, steroids, tannins, saponins, and alkaloids. Flavonoids and tannins, which contain phenolic groups, are known to act as reducing agents in the synthesis process, assisting in the reduction of Ag^+ ions to silver nanoparticles (Ag^0) and stabilizing the particles by inhibiting excessive aggregate growth, resulting in smaller particle sizes. This smaller particle size implies an increased active surface area, which is crucial for biological activity [12].

In addition to their role in the synthesis process, these secondary metabolites also contribute to antibacterial activity [13]. Flavonoids and tannins are known to damage bacterial cell membranes and disrupt essential enzyme function, while saponins can increase bacterial cell membrane permeability. The combination of secondary metabolite activity with the antimicrobial properties of Ag/CeO_2 nanoparticles produces a synergistic effect, strengthening antibacterial mechanisms through cell wall damage, the release of silver ions, which are toxic to microorganisms, and the formation of reactive oxygen species (ROS), which can damage bacterial cellular components [9]. The phytochemical content of noni leaves is in accordance with research [6, 13].

Characterization result

Characterization using XRD was aimed at obtaining the 2θ angle position, peak intensity, and crystal phase and determining the success of the nanocomposite synthesis. **Figure** 2 displays the diffractogram of the Ag/CeO_2 nanocomposite synthesized using noni leaf extract.

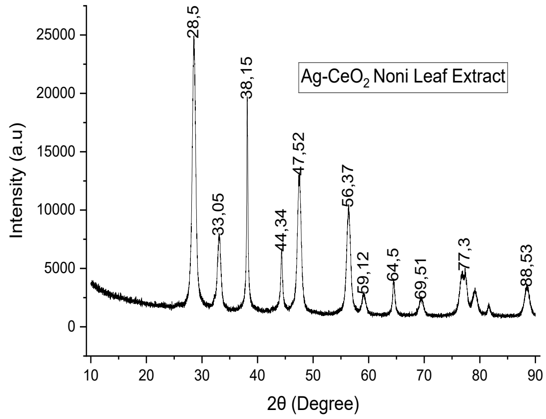

Figure 2. Diffactogram Ag/CeO_2 nanocomposite synthesized using noni leaf extract.

Based on JCPDS standard data No. 04-0783, silver (Ag) has four characteristic diffraction peaks at 2θ angles: 38.14° (111), 44.27° (200), 64.18° (220), and 78.24° (311) [14]. Meanwhile, JCPDS data No. 34-0394 shows that CeO_2 has six characteristic peaks at 2θ angles: 28.71° (111), 33.18° (200), 47.62° (220), 56.37° (311), 59.08° (222), and 69.52° (400) [15]. The diffractogram analysis results in Figure 2 show that the Ag/CeO_2 material synthesized using noni leaf extract has peaks at 2θ angles of 28.5°, 33.05°, 38.15°, 44.34°, 47.52°, 56.37°, 59.12°, 64.5°, 69.51°, 77.3° and 88.53°. The presence of these peaks indicates excellent agreement with the standard diffraction patterns of Ag and CeO_2, confirming the successful synthesis of the Ag/CeO_2 nanocomposite using noni leaf extract.

The diffractogram in **Figure** 2 does not show additional peaks with low intensity other than the characteristic peaks of Ag and CeO_2, this does not rule out the possibility of the presence of organic compounds from noni leaf extract. Phytochemical components such as flavonoids, tannins, and saponins tend to be amorphous and only adhere thinly to the surface of the nanoparticles, so they do not produce sharp Bragg peaks in XRD analysis. Therefore, the presence of these organic compounds is usually detected through complementary characterization techniques such as FTIR [16].

Furthermore, the average crystallite size of the Ag/CeO_2 nanocomposite was calculated using the Debye-Scherrer equation and found to be 15.28 nm. This value indicates that the synthesized material is on the nanometer scale, so it has the potential to have better antibacterial activity and functional properties than micrometer sized materials.

SEM characterization was performed to observe the morphology of Ag/CeO_2 synthesized using noni leaf extract. The results of the nanocomposite characterization using SEM are shown in **Figure** 3.

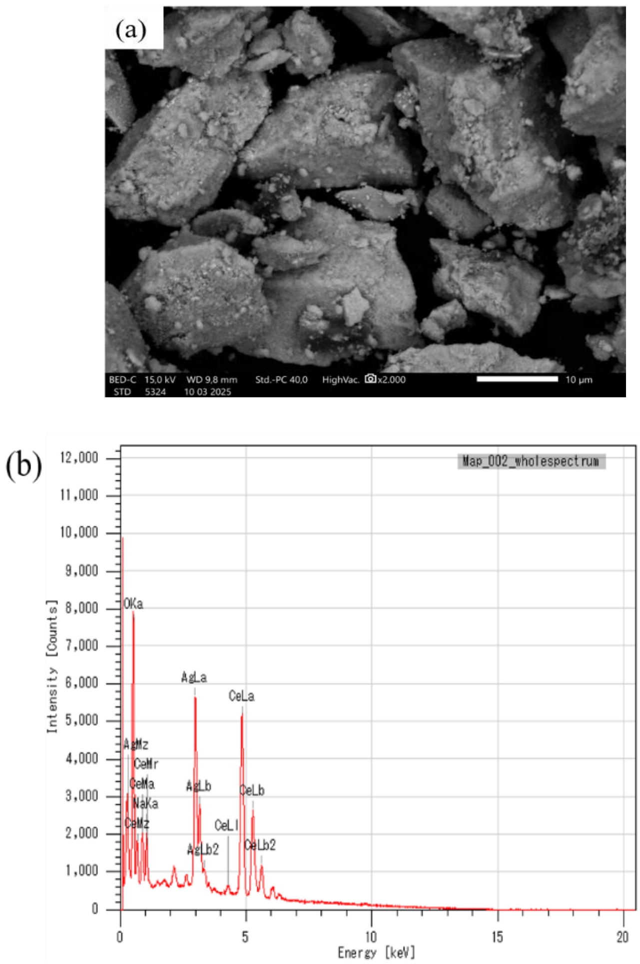

Figure 3. Morphological Ag/CeO_2 nanocomposite synthesized using noni leaf extract

Morphological characterization using Scanning Electron Microscopy (SEM) in **Figure** 3 (a) shows that the Ag/CeO_2 nanocomposite has irregular particle shapes with rough surfaces and tends to agglomerate [17]. This morphology can increase the specific surface area of the material, thereby increasing the contact area with bacteria. High surface area is an important factor in antibacterial activity, as it facilitates the release of Ag^+ ions and direct interaction of particles with bacterial cell membranes [18].

The elemental composition analysis using EDX in **Figure 3 (b)** shows the presence of Ce, O, and Ag as the main components. Quantitative EDX data show that Ce dominates the composition with a mass of 58.52%, followed by Ag at 24.39%, and O at 14.37%. This relatively high silver content is important, considering that Ag^+ is known to have a strong antibacterial mechanism through cell membrane damage, the formation of reactive oxygen species (ROS), and interactions with bacterial DNA that inhibit the replication process. Meanwhile, CeO_2 plays a role not only as a supporting matrix, but is also capable of undergoing Ce^{3+}/Ce^{4+} redox, which contributes to the generation of ROS, thus increasing the synergistic effect with Ag [19].

The presence of significant amounts of oxygen indicates the formation of a stable oxide structure, potentially supporting the release of active ions during interaction with bacteria [20]. Minor elements such as Na (2.72%) likely originate from residual noni leaf extract, which acts as a reducing agent in the synthesis but does not affect the material's primary activity. The absence of Cl and C indicates that organic residues have been reduced, making the resulting material purer and ready for use in antibacterial activity tests.

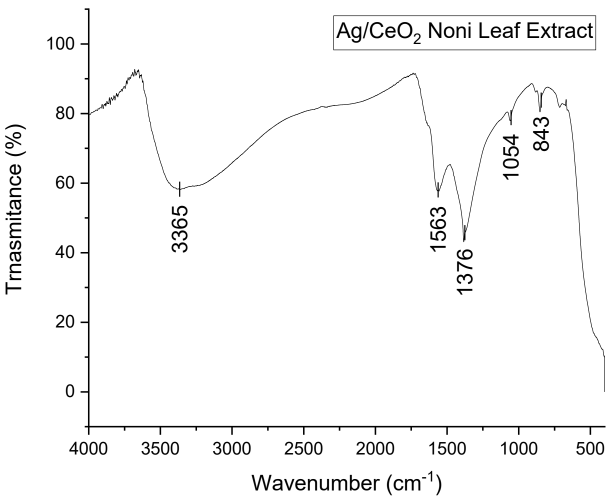

Figure 4. Spectrum of Ag/CeO_2 synthesized using noni leaf extract

Characterization using FTIR to determine the functional groups in Ag/CeO_2 synthesized using noni leaf extract. **Figure** 4 shows the FTIR spectrum of Ag/CeO_2.

The spectrum in **Figure** 4 shows a broad absorption at wave number 3365 cm^{-1} which is a characteristic of the hydroxyl group (O-H) derived from noni leaf extract. The absorption bands at 1562 cm^{-1} and 1053 cm^{-1} show various typical bands of functional groups found in noni leaf extract, such as aromatic C=C stretching, alcohol C-O and esters [21]. Metal oxide characteristics appear at wave numbers 600-850 cm^{-1}, data obtained at wavenumber 843 cm^{-1} in the spectrum indicates the presence of Ce-O in the compound. The Ag element was not detected because Ag is a pure element that will be difficult to detect in FTIR.

Antibacterial activity

The antibacterial activity of the Ag/CeO_2 nanocomposite synthesized using noni leaf extract is shown in **Figure** 5, while the diameter value of the inhibition zone produced against each bacterial strain can be seen in more detail in **Table** 2.

The test results showed that the nanocomposite could inhibit the growth of both types of bacteria with the diameter of the inhibition zone increasing with increasing concentration. At a concentration of 1000 mg/L, the inhibition zone was still relatively small (6–7 mm), while at a concentration of 5000 mg/L, the activity increased significantly, with the inhibition zone reaching 8 mm (moderate category) against both *S. aureus* and *E. coli*. Although this value is still lower than the positive control amoxicillin (13.5 mm), these results indicate that the $Ag\text{-}CeO_2$ nanocomposite material has potential as an alternative nanomaterial based antibacterial agent.

The antibacterial mechanism of the $Ag\text{-}CeO_2$ nanocomposite is related to the synergistic properties between CeO_2 and Ag. CeO_2 nanoparticles are known to produce reactive oxygen species (ROS) that can damage proteins, nucleic acids, and disrupt the function of bacterial cell organelles, thus causing cell death [22]. Meanwhile, the presence of Ag in the nanocomposite not only increases the specific surface area, but also plays a role in the release of Ag^+ ions which have strong antibacterial effects. Ag^+ ions can bind to thiol groups on cell membrane proteins, disrupting membrane permeability, and interacting with bacterial DNA which inhibits cell replication. The combination of these two mechanisms strengthens the antibacterial effect of the nanocomposite.

Other factors that influence antibacterial effectiveness are the type of bacteria, the diffusion capacity of the material, and the presence of residual metabolites from the noni leaf extract that may contribute to the inhibition of bacterial growth. The structure of the cell wall also plays an important role, where Gram-positive bacteria (*S. aureus*) have a thicker peptidoglycan layer, while Gram-negative bacteria (*E. coli*) have an additional outer membrane that can limit the diffusion of nanostructures into the cell [4]. This explains the relative differences in inhibition zones at several test concentrations.

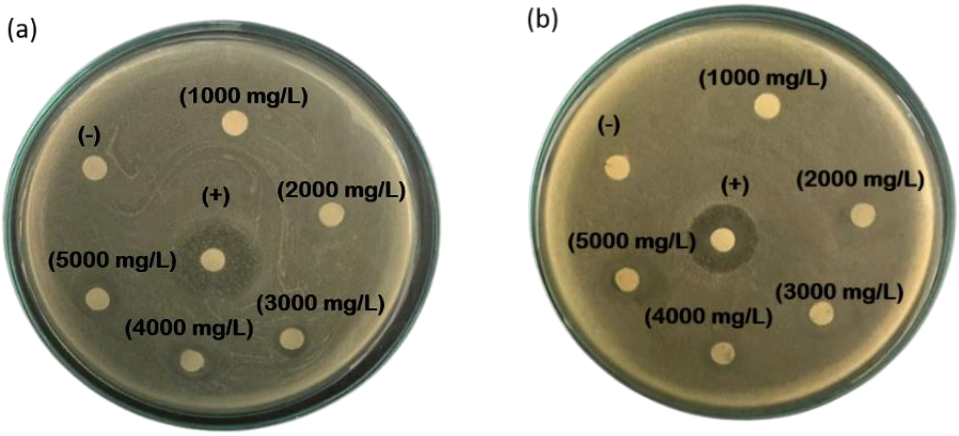

Figure 5. Ag/CeO_2 nanocomposite synthesized using noni leaf extract for antibacterial test of (a) *S.aureus*, (b) *E.coli*

Table 2. Ag/CeO_2 nanocomposite synthesized using noni leaf extract for antibacterial test of *S.aureus* and *E.coli*

Nanocomposite Concentration (mg/L)	***S. aureus* (mm)**	***E. coli* (mm)**
Amoxicillin Positive Control (1000 mg/L)	13.5 ± 0.0	13.5 ± 0.0
DMSO Negative Control (1000 mg/L)	6.0 ± 0.0	6.0 ± 0.0
Nanocomposite 1000 mg/L	6.0 ± 0.0	6.3 ± 0.2
Nanocomposite 2000 mg/L	6.1 ± 0.2	6.1 ± 0.2
Nanocomposite 3000 mg/L	6.3 ± 0.5	7.0 ± 0.5
Nanocomposite 4000 mg/L	7.1 ± 0.5	6.5 ± 0.5
Nanocomposite 5000 mg/L	8.1 ± 0.5	8.0 ± 0.5

CONCLUSION

Ag/CeO_2 nanocomposites were successfully synthesized using noni leaf extract through a green synthesis method, with an average crystallite size of 15.28 nm. XRD, FTIR, and SEM-EDX characterizations confirmed the successful synthesis and material composition. Antibacterial tests showed activity against S. aureus and E. coli, with a maximum inhibition zone of 8 mm at a concentration of 5000 mg/L. This activity was supported by the synergy of Ag^+ ion release and ROS formation by CeO_2. These results confirm the potential of Ag/CeO_2 as an environmentally friendly antibacterial agent.

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