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Analysis on Silica and Graphene Nanomaterials Obtained From Rice Straw for Antimicrobial Potential

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ABSTRACT

This study focuses on the encapsulation of silica and graphene nanoparticles and their potential applications. The encapsulation enhances the properties and effectiveness of these nanoparticles, with silica providing stability and graphene contributing to high surface area and electrical conductivity. Characterization of silica-graphene nanoparticles was conducted using various techniques including High Power Microscope (HPM), Scanning Electron Microscope (SEM), Energy-dispersive X-ray spectroscopy (EDS), and 3D Nano Profiler. The antimicrobial activity of silica, graphene, and silica-graphene nanoparticles was evaluated using a disc diffusion assay against E. coli and B. subtilis at varying concentrations. Results showed significant antimicrobial activity, with the inhibition zone being directly proportional to the concentration. Silica-graphene nanoparticles demonstrated higher efficacy against E. coli compared to B. subtilis, attributed to differences in cell wall structure. Statistical analysis using ANOVA confirmed significant differences in antimicrobial activity among the tested components.

Keywords: *Antimicrobial activity, Morphological and element chemical availability, Synthesis, Silica-Graphene nanoparticle*

1. INTRODUCTION

In recent times, there is a growing interest in finding better ways to create nanoparticles in a way that good for the environment. With the increasing need to tackle global environmental problems, researchers are now looking into using materials that can be renewed and waste products to develop new, environmentally friendly nanomaterials [1–5]. One such potential candidate is rice straw, an abundant agricultural residue that is rich in carbon-based compounds. This study endeavours to harness the untapped potential of rice straw ash to synthesize a novel combination of silica and graphene nanoparticles through a green synthesis approach. By employing this eco-friendly method, the environmental impact associated with conventional nanoparticle synthesis processes can be significantly reduced, contributing to a greener and more sustainable future.

To ensure the success of the encapsulated nanomaterial, thorough characterization of the synthesized silica-graphene nanoparticles will be conducted. Advanced techniques, such as scanning electron microscopy (SEM), will be employed for morphological analyses, enabling examination of particle size, shape, and distribution. Furthermore, a High-Power Microscope (HPM), a sophisticated imaging tool, will play a crucial role in nanoparticle analysis. Specifically designed for high-resolution imaging, HPMs facilitate the observation of nanoparticles at the nanoscale level, providing essential insights into their structural features. This meticulous characterization process is integral to validating the quality and properties of the synthesized nanomaterial, ensuring its efficacy for intended applications [6].

Moreover, the research will delve into the crucial evaluation of the antimicrobial activity of the synthesized silica-graphene nanoparticles. The antimicrobial potential of the composite will be subjected to rigorous testing against various microorganisms, including gram-negative Escherichia coli and gram-positive Bacillus subtilis. This assessment is of paramount importance, as it opens up potential applications in diverse sectors, such as medicine, agriculture, and water treatment, where the demand for effective antimicrobial agents is ever-increasing [7]. Furthermore, the antimicrobial activity of the nanoparticles will be tested at various concentrations to determine the minimum inhibitory concentration (MIC), which is the lowest concentration at which the nanoparticles effectively inhibit bacterial growth. Determining the MIC is crucial in understanding the

nanoparticles potency and optimizing their use as antimicrobial agents [8].

Overall, the encapsulation of silica and graphene nanoparticles derived from rice straw ash using a green synthesis approach holds great promise for sustainable nanomaterial development. The successful realization of this research could revolutionize nanoparticle synthesis practices, demonstrating the feasibility of eco-friendly and resource-efficient methods. Furthermore, this investigation has the potential to contribute significantly to waste management strategies by transforming agricultural waste into valuable nanomaterials with antimicrobial properties. By addressing the pressing need for green and effective antimicrobial agents, this study aims to make significant strides towards a cleaner, healthier, and more sustainable world.

This research aims to synthesize silica-graphene nanoparticles from rice straw ash, employing an eco-friendly approach. Advanced characterization techniques, including scanning electron microscopy and High-Power Microscopy, will scrutinize particle morphology. The study will rigorously evaluate antimicrobial efficacy against Escherichia coli and Bacillus subtilis, determining the minimum inhibitory concentration for optimal performance. Exploring applications in medicine, agriculture, and water treatment, the research contributes to the growing demand for effective antimicrobial agents. By addressing these objectives, the study seeks to revolutionize nanoparticle synthesis, advance sustainable nanomaterial development, and contribute to waste management for a cleaner, healthier, and more sustainable global future.

2. MATERIAL AND METHODS

2.1. Collection and Preparation of Rice Straw Bioproduct

The rice straw will be obtained from a specific location in Tanjong Karang Selangor, Malaysia (1186-A Jalan Menanti 3 Kampung Sungai Tengi Kiri 4500) with the coordinates 3.435185722130434 N and 101.16938803104138°E. In the initial treatment step, sodium hydroxide and controlled combustion methods were utilized. Once the raw materials are collected, the rice straw undergoes a washing process with deionized water and is subsequently sun-dried for three days. The drying process occurs from 11 a.m. to 5 p.m. After the rice straw is thoroughly cleaned and dried, it is subjected to controlled burning until it transforms into ash.

2.2. Synthesis of Silica Nanoparticle

Rice straw ash was mixed with sodium hydroxide solution and heated at 100 degrees Celsius for four hours. The mixture was then filtered, and sulfuric acid was added to adjust the pH to 7.0. The mixture was stirred for 18 hours, and two different layers of gel formed. The mixture was centrifuged, and the resulting particles were collected. This process was repeated three times, once with ethanol and twice with distilled water. The pellet was dried in an oven at 80 degrees Celsius for 30 minutes, and then ground into a powder [9].

2.3. Synthesis of Graphene Nanoparticle

Rice straw ash and potassium hydroxide were mixed and heated at 700 degrees Celsius for two hours. The mixture was then combined with deionized water and stirred for six hours The excess potassium hydroxide was removed by washing the sample with distilled water and centrifuging. The sample was then dried in a microwave at 150 degrees Celsius for 24 hours. It is now ready for further analysis [10].

2.4. Encapsulation of Silica and Graphene Nanoparticle

A mixture was prepared by combining 1 mg/ml of silica and graphene with 2% (3-Aminopropyl) triethoxysilane (APTES) in 30% ethanol. The mixture was placed in a porcelain crucible in the form of a brick and sealed with a crucible cap. It was then incubated for 1 hour. In a separate step, the mixture was combined with 1 mg/ml of graphene in the presence of distilled water. The resulting sample was incubated for 1 hour. To remove excess ethanol and APTES, the sample was washed with distilled water before being centrifuged at 6000 x g for 10 minutes. Subsequently, the sample was dried using a microwave at 150 °C for 24 hours, making it ready for further investigation [11].

2.5. The Silica-Graphene Nanoparticle Characterization

2.5.1 High Power Microscope (HPM)

The surface and elemental structures of silica-graphene nanoparticles were analyzed using HPM. To perform the analysis, a small number of silica-graphene particles were added to 50 mL of purified water and stirred. Then, 30 mL of the solution was applied to a clean chip and observed from a distance of 8 mm, using an accelerating voltage of 5 kV [12].

2.5.2 Scanning Electron Microscope (SEM)

To prepare the silica-graphene nanoparticle sample, initial steps involve cleaning the sample, applying an electrically conductive material to cover it, and placing it on a sample stand. Ensuring conductivity is important for the movement of electrons within the silica-graphene nanoparticle. Subsequently, an electron beam is generated, with the energy level adjustable between 1 and 40 keV. The electron beam is then directed across the surface of the sample. As the electron beam interacts with the atoms in the sample, various signals are produced. These signals provide information that can be used to create an image of the sample's surface [13].

2.5.3 Energy-Dispersive X-ray Spectroscopy (EDS)

To enhance conductivity and minimize charging effects during imaging, a layer of metallic material such as gold or carbon was applied on the silica-graphene nanoparticle. Once the nanoparticle was prepared, it was securely placed on a sample stand to ensure stability. The sample holder, containing the nanoparticle, was then inserted into the EDS tube. A vacuum was established to create optimal imaging conditions. The energy of the electron beam typically ranged between 15 and 30 keV. The electron beam was directed across the sample, and the resulting signals were recorded for analysis and observation [13].

2.5.4 3D Nano Profiler

The 3D Nano profiler captured an image displaying the surface of a silica-graphene nanoparticle, which serves as a sensing material. In the image, variations in color represent different surface heights. Darker areas indicate higher regions, while lighter areas indicate lower regions. To create this image, a small amount of silica-graphene powder was mixed with 50 µl of pure water. After complete dissolution of the powder in water, 30 µl of the resulting mixture was added to sterile water. The image was then obtained using a 3D Nano profiler, with a working distance of 8 mm [14].

2.5.5 Antimicrobial Activity

The agar well diffusion method was used to assess the antibacterial activity of silica-graphene nanoparticles against the oral pathogens Escherichia coli (E. coli) and Bacillus subtilis (B. subtilis). Nutrient agar powder was prepared and poured into petri dishes, and stock cultures of the microorganisms were established. Silica-graphene nanoparticles were synthesized and serially diluted for testing. A positive control was included using a commercial antibiotic, ampicillin (50 mg/ml), for both E. coli and B. subtilis. The antibacterial activity was evaluated by measuring the zones of inhibition on the agar plates after incubation. The study aimed to compare the activity of the nanoparticles against gram-positive and gram-negative bacteria. The experimental procedure and disc diffusion assay were described, and the results were assessed based on the size of the inhibition zones [14]-[15].

3. RESULTS AND DISCUSSION

3.1 Encapsulation of Silica and Graphene Nanoparticle

Figure 1 shows that the combination of graphene and silica into nanoparticles enhances their properties and effectiveness. Silica provides stability, while graphene contributes a high surface area and excellent electrical conductivity. This synergistic combination opens numerous potential applications. One such hybrid nanoparticle is silica-graphene, which combines the characteristics of both materials. Graphene, consisting of a single layer of carbon atoms, is highly conductive, while silica, a non-conductive substance, is commonly used as a coating. The combination of silica and graphene makes silica-graphene nanoparticles promising materials for various applications, including catalyst, biomedicine, and electronics [17].

Figure 1. The Result of Encapsulation Silica and Graphene Nanoparticle.

3.2 The Silica-Graphene Nanoparticle Characterization

3.2.1 High Power Microscope (HPM)

Using a high-powered microscope (HPM), the structure of effluent silica-graphene was determined. The solution of silica-graphene was deposited on a wafer, and its structure was analyzed using HPM. Figure 2 depicts the image of effluent silica-graphene at various magnifications under bright-field (BF) and dark-field (DF) illumination. It displays the structure of the algal extract solution [18]. As shown in Figures 2, using high-pressure microscopy, the surface morphology of synthesized silica-graphene nanoparticles was characterized. Initial disclosure of this morphology included all surface structures of the original silica nanoparticles on silicon substrates at four different enlargements (5x, 10x, and 20x). Explicit images of silica-graphene nanoparticles were observed at these magnifications. According to the images, the particles have a crude arrangement with various irregularly structured particles [14].

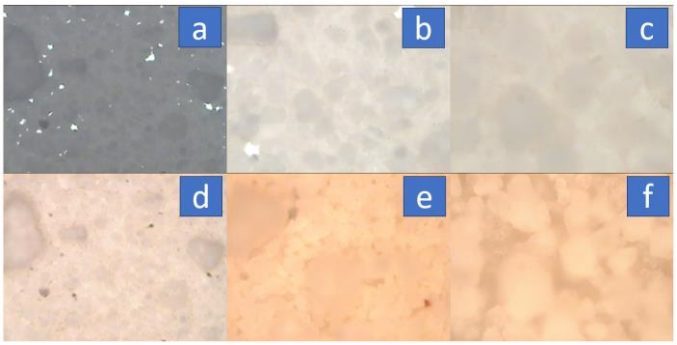

Figure 2. The image of effluent silica-graphene at various magnifications under bright-field (a,b,c) and dark-field (d,e,f); (a,d: 5x; b,e:10x; c,f:20x) under high power microscope.

3.2.2 Scanning Electron Microscope (SEM)

Scanning electron microscopy (SEM) was used to investigate the size, shape, morphology, and surface chemistry of silica-graphene nanoparticles. The SEM micrographs revealed highly crystalline, aggregated,

spherical silica-graphene particles of varying diameters. The particles were polycrystalline, meaning that they were made up of many small crystals. The crystals had a face-centered cubic (FCC) structure, which is the most common structure for metals at the nanoscale level. The particles tended to nucleate and grow on doubly and multiply doubly twinned particles, which are particles that have been split into two or more identical halves. The surfaces of the particles were bordered by (111) facets, which are the lowest energy facets in a fcc crystal [19]. Silica-graphene nanoparticles tend to agglomerate due to their high surface tension. The high surface tension of these nanoparticles means that they have a strong tendency to attract each other and form larger particles. This is because the particles have a high energy due to their small size, and they will try to minimize their energy by forming larger particles. [20].

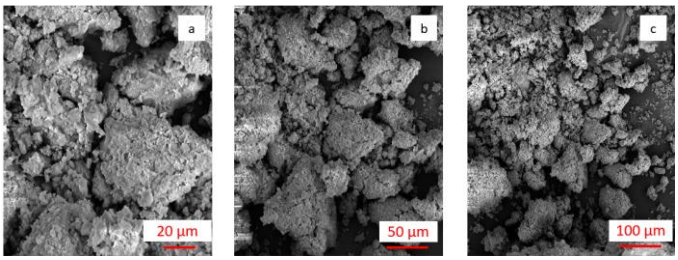

Figure 3. SEM image of biosynthesized silica-graphene nanoparticle. Magnification (a) 100x; (b) 500x and (c) 250x.

3.2.3 Energy-Dispersive X-Ray Spectroscopy (EDS)

Figure 4 displays the EDS results for the atomic percentages of silicon, oxygen, and carbon in rice straw ash, which are 12.00%, 40.63%, and 39.58%, respectively. It demonstrated that the percentage of elemental composition from our research findings closely align with previous studies. Thus, it is demonstrated that the synthesized silica nanoparticles contain an abundance of silica-graphene nanoparticle originality [21].

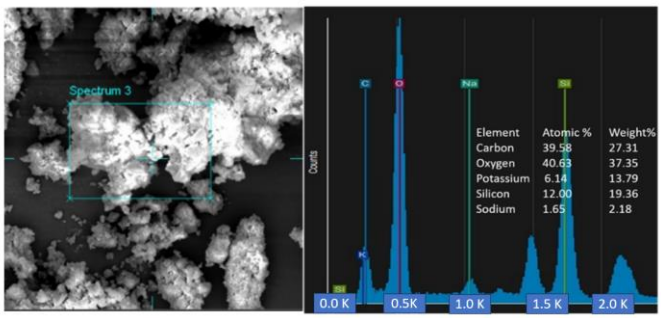

Figure 4. EDS for Silica-graphene.

3.2.4 3D Nano Profiler

Figure 5 is a representation of a 3D Nano-profiler image that offers valuable insight into the surface characteristics and physical properties of the silica-graphene composite. The variation in color represents the height differences across the surface, with various hues denoting different height levels[22]. Figure 5a demonstrates that the composite of silica and graphene has a height range of 80,5 nm between its lowest and topmost points. This information provides a comprehension of the surface's topographical variations. In addition, the average surface height of the silica-graphene composite is determined to be 909.40 nm, indicating the surface profile. In Figure 5d, the surface irregularity and undulation of the silica-graphene composite are analyzed in greater detail. The 103.0 nm roughness value indicates the presence of surface irregularities and height variations on a lesser scale. This irregularity is a significant property because it can affect the material's properties and interactions with its surroundings. In addition, the wave height of 130726.3 nm indicates larger-scale surface undulations and deviations. These surface properties are significant because they can influence the functionality and efficacy of the silica-graphene composition [23]-[24]. The presence of surface irregularity and undulations can impact adhesion, mechanical stability, and optical properties, among others. In order to evaluate the prospective applications of the silica-graphene composite, it is necessary to comprehend and characterize these physical properties. Importantly, the discussion of the results presented in Figure 5 should be supported by additional data analysis and interpretation. In addition, it is necessary to consider the experimental conditions, sample preparation, and measurement parameters to ensure accurate and trustworthy conclusions regarding the surface properties of the silica-graphene composition [19].

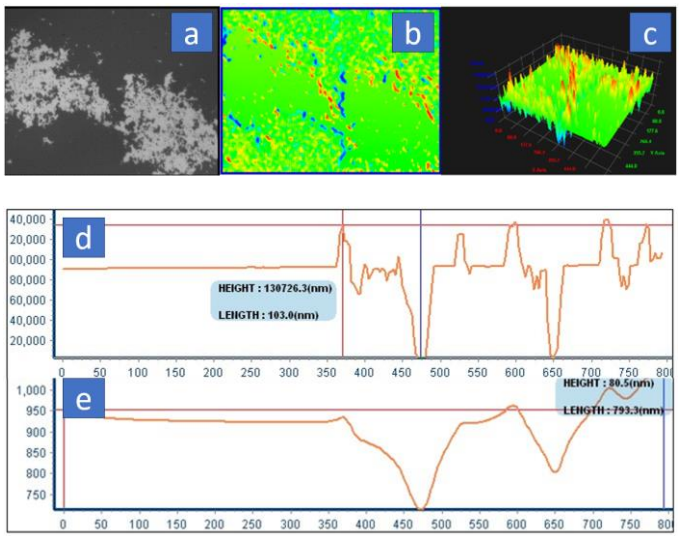

Figure 5. 3D nano profiler imaging 3D view (a) The silica-graphene surface was smooth and sharp as can see in the image (b); the 2D image; (c) 3D image; (d) data of roughness; (e) data of waviness.

3.3 Antimicrobial Activity

The antibacterial activity of silica, graphene, and silica-graphene nanoparticles was investigated using a disc diffusion assay. The zone of inhibition, which is the area around the disc where bacteria are unable to grow, was measured to assess the susceptibility of the microorganisms. The components were also examined at varying concentrations, with silica, graphene, and silica-graphene nanoparticles being tested at 1 µg/100 L, 0.5 µg/100 L, 0.25 µg/100 L, 0.125 µg/100 L, and 0.063

µg/100 L. This allowed for further investigation into the effect of concentration variation on the inhibitory zone. Figure 6 illustrates the antibacterial activity of the components under investigation against E. coli and B. subtilis at varying concentrations.

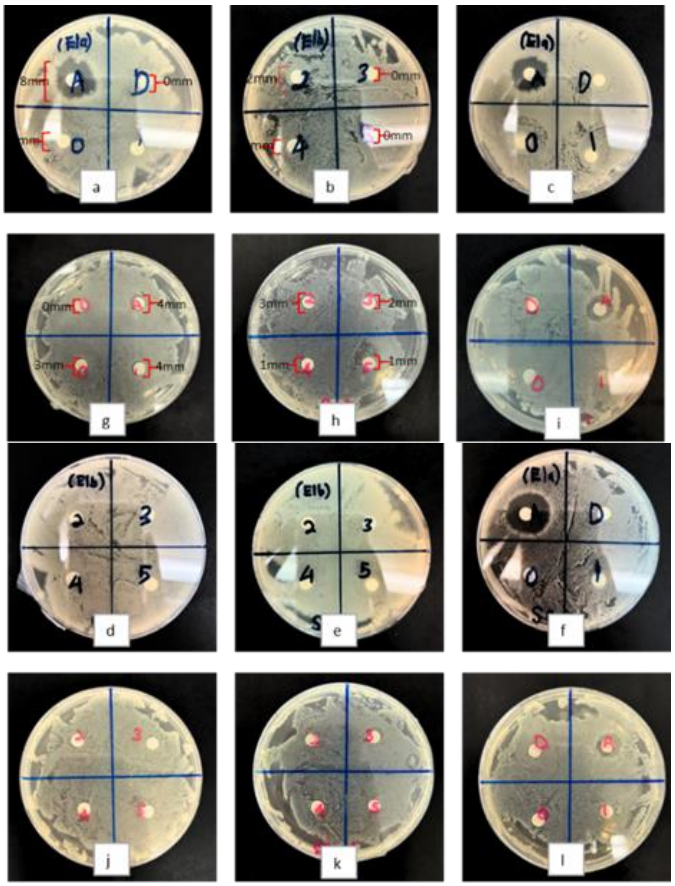

Figure 6. Illustrates the antibacterial activity of the components under investigation against E. coli; (a) silica; (b) silica ; (c) graphene; (d) graphene; (e) silica-graphene; (f) silica-graphene and B. subtilis (g) silica; (h) silica ; (i) graphene; (j) graphene; (k) silica-graphene; (l) silica-graphene. Antimicrobial activity of the tested components (Amoxicillin (A), Distilled water (D), silica/graphene/silica-graphene (0,1,2,3,4,5) with concentration of silica or graphene or silica-graphene 1mg/100µL, 0.5 mg/100µ and 0.25 mg/100µL for (2,3,4,5).

The antibacterial activity of silica, graphene, and silica-graphene nanoparticles was studied using a disc diffusion assay. The zone of inhibition, which is the area around the disc where bacteria are unable to grow, was measured to determine the susceptibility of the microorganisms. The components were also tested at different concentrations: 1 µg/100 L, 0.5 µg/100 L, 0.25 µg/100 L, 0.125 µg/100 L, and 0.063 µg/100 L. This allowed for further investigation into the effect of concentration variation on the inhibitory zone. Figure 6 shows the antibacterial activity of the components under investigation against E. coli and B. subtilis at varying concentrations [25]. The cell wall is a very important part of a bacterial cell. It protects the cell from harmful substances and helps to give the cell its shape. The cell wall is also a target for antibiotics. Gram-positive bacteria have a thick cell wall that lacks an outer lipopolysaccharide membrane. This makes them more susceptible to damage from silica-graphene nanoparticles than gram-negative bacteria, which have a thinner cell wall with an outer lipopolysaccharide membrane [25].

As refer to table 1, the inhibition zone is the largest at the highest concentration (1 mg/100 L) for all tested components. This indicates that the inhibition zone is directly proportional to the concentration. In other words, as the concentration increases, so does the inhibition zone. This is because E. coli has a thin layer of peptidoglycan between its inner and outer lipid membranes, while B. subtilis has a thicker cell wall. The silica-graphene nanoparticles are able to disrupt the cell wall of E. coli more easily than the cell wall of B. subtilis, which is why the inhibition zone is larger for E. coli [26]. The thin peptidoglycan layer of E. coli makes it more susceptible to the effects of silica-graphene nanoparticles than the denser peptidoglycan layer of B. subtilis. This is because the nanoparticles are able to diffuse more easily through the cell wall of E. coli, disrupting its cellular function and inhibiting its growth.

At a concentration of 1 mg/100 µL, the average inhibition zone of biosynthesized silica, graphene, and silica-graphene in E. coli is 3 mm, 1 mm, and 3 mm, respectively. In B. subtilis, the inhibition zones are 2 mm, 3 mm, and 2 mm, respectively. This shows that the nanoparticles are more effective against E. coli than B. subtilis [16]. The positive control amoxicillin demonstrated the most antimicrobial activity against E. coli, with an inhibition zone of 5 mm. Silica and silica-graphene also showed some antimicrobial activity, with inhibition zones of 3 mm and 2 mm, respectively. Graphene showed no antimicrobial activity. The limited penetrating power of silica and silica-graphene allows them to access the bacterial cell wall more easily, which is why they are more effective against E. coli than B. subtilis. At a low concentration of 1 mg/100 µL, the microbial inhibition of biologically synthesized silica and silica-graphene demonstrated an inhibition zone with a low antimicrobial potential. This suggests that higher concentrations of the nanoparticles may be needed to achieve effective antimicrobial activity [27].

ANOVA is a statistical test that can be used to determine if the means of three or more groups are significantly different. The P-value is a measure of the statistical significance of the difference between the groups. A P-value of less than 0.05 indicates that the difference is statistically significant. In the study cited in the search results, the P-values for silica-graphene, silica, graphene, and amoxicillin were all less than 0.05. This indicates that the antimicrobial activity of each component at the specified concentration was significantly different from the others [16]. The ANOVA test is a useful tool for analyzing differences in group means and determining the impact of independent variables on the dependent variable. It can help to understand the efficacy of antimicrobial agents and their potential clinical application.

Table 1 Measurement of silica, graphene, silica-graphene, amoxicillin and distilled water.

Components	Concentration (mg/100µL)	Zone of Inhibition (mm), in E. coli and B. subtilis	
		B. subtilis	E. coli
Silica	1	3.0 (±1.33)	2.0 (±1.33)
	0.5	3.0 (±0.12)	2.0 (±0.67)
	0.25	3	1.0 (±0.33)
	0.125	2.0 (±0.7)	0
	0.063	2.0 (±0.67)	0
	0.0313	1.0 (±0.33)	0
Amoxicillin	50	2.0 (±2.0)	4.0 (±1.5)
Graphene	1	1	3.0 (±0.67)
	0.5	0	2 (±0.67)
	0.25	0	0.6
	0.125	0	0
	0.063	0	0
	0.0313	0	0
Amoxicillin	50	3.0 (±2.0)	5.0 (±1.5)
Silica-Graphene	1	3.0 (±0.6)	2.0 (±0.67)
	0.5	2	2.0 (±0.5)
	0.25	1(±0.5)	2.0 (±0.3)
	0.125	1 (±0.45)	1.0 (±0.3)
	0.063	1 (±0.32)	1
	0.0313	0	0
Amoxicillin	50	2.0 (±2.0)	4.0 (±1.5)
Distilled Water	0	0	0

CONCLUSION

In summary, our study extensively investigated the antimicrobial potential of silica, graphene, and silica-graphene nanoparticles, yielding crucial insights through quantitative measurements. The disc diffusion assay unveiled distinct inhibitory zones around the discs at concentrations ranging from 1 µg/100 L to 0.063 µg/100 L. Remarkably, the highest concentration (1 mg/100 L) showcased the most substantial inhibition zones for all components against both E. coli and B. subtilis. The varied efficacy against the two bacterial strains can be attributed to their unique cell wall structures. Silica and silica-graphene nanoparticles exhibited more pronounced inhibitory effects on E. coli, characterized by a thinner peptidoglycan layer, compared to B. subtilis with a denser cell wall. The statistically significant differences in antimicrobial activity, highlighted by the ANOVA test, underscore the potential clinical applications and effectiveness of these nanoparticles. Further investigations are essential to fine-tune concentrations for heightened antimicrobial efficacy and increased clinical significance.

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