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RESEARCH ARTICLE

SUSTAINABLE PRODUCTION of AgO:FeO NANOCOMPOSITES: STRUCTURAL, OPTICAL, AND ANTIMICROBIAL ANALYSIS

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Abstract. The green synthesis of metal oxide nanoparticles has attracted considerable interest owing to its eco-friendly, cost-effective, and sustainable nature. Silver oxide (AgO) and iron oxide (FeO) nanoparticles exhibit good antibacterial and optical properties and their coalescence into nanocomposites may enhance biological activity through synergistic effects. This work contains the green synthesis and characterization of silver oxide/iron oxide (AgO:FeO) nanocomposites by a green chemistry strategy. The structural, morphological, elemental compositional and optical properties were investigated using X-ray diffraction (XRD), field emission scanning electron microscope (FESEM), energy dispersive x-ray (EDX), Fourier transform infrared spectroscopy (FTIR), and UV-visible spectroscopy. Analysis with XRD patterns revealed that pure crystalline AgO and FeO phases had formed without any detectable impurities. Nanoparticles showed mild agglomeration with average diameter ca 164 nm in FESEM imaging. The FTIR spectra analyses gave evidence of bio-organic functional groups originating from the plant extract, in addition to metal–oxygen bonds that signifying successful green synthesis route. Characteristic surface plasmon resonance that confirmed the formation of nanocomposite was seen from UV–visible spectroscopy. The antibacterial activity of the AgO:FeO nanocomposites was evaluated by mean of the agar well diffusion method against a broad spectrum of pathogenic microorganisms, such as Gram-positive bacteria (*Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis)*), Gram-negative bacteria (*Escherichia coli* (*E. coli*) *and Klebsiella species*)). The nanocomposites exhibited significant antibacterial activity with the largest inhibition (23.67 mm) against *S. epidermidis*. The AgO: FeO nanocomposite showed synergistic antibacterial properties which were comparable with, or even greater than those of the individual AgO and FeO nanoparticles. This improvement is mainly due to high surface and enhanced ROS production. These findings highlight the potential of AgO:FeO nanocomposites synthesized through a biosynthesized green chemistry process as effective antimicrobial agents for medicinal applications.

Keywords: AgO:FeO nanocomposite, green synthesis, *Anethum graveolens*, antimicrobial activity.

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1. INTRODUCTION

Bacterial infections are still a major public health problem all over the world with high contribution to global mortality. Compounding this threat is the increasing sophistication of infection yreatment coupled with rising antibiotic resistance. In addition, the health care costs associated with bacterial infections are enormous, costing billions of dollars yearly [1,2]. An example of a new strategy is that metal nanoparticles are integrated into drug delivery systems to improve antimicrobial treatments [3]. However, the interactions between nanoparticles and heavy metal during biological detoxification processes are still not well investigated. Nanotechnology is based on the science of particles smaller than 1 to 100 nanometers and have been used effectively in commercial products like coatings, electronics, energy as well as sophisticated pharmaceutical reasons. The potential of nanoparticles is more pronounced and studied in medicine and pharmacy [4].

With increasing demand from several industries, there is high interest in designing and synthesizing new metal oxide nanocomposites with improved physicochemical properties. They are used in the fields of biomedicine, energy storage, optoelectronics and catalysis [5]. Silverbased materials, especially silver oxide (AgO), is becoming a favored candidate due to its remarkable antifungal and antibacterial bioactivity as well as catalytic activity [6,7]. Recent studies have shown that the composition of the biological source from which silver nanoparticles are derived has a crucial role in its toxicity and antimicrobial effectiveness [8]. Due to the synergetic action of each phase, AgO exhibits any strengthening when matched with various metal oxides. The existing methods for the preparation of AgO nanocomposites are hydrothermal, chemical precipitation, solvothermal and plasma [9]. Green and laser-assisted synthesis approaches have been used for hybrid organic–inorganic Metal oxide nanoparticles (ZnO) [10]. Yet most of these strategies depend on toxic chemicals and reductants which are hazardous to the environment and biology. Thus, synthesis methods are increasingly guided by the principles of green chemistry defined by Anastas and Warner focusing on reducing hazardous substances with increased efficiency of processes [11].

Recent advances in the production of green nanoparticles suggest that biological systems from bacteria, fungi, algae and plants can be used as an environmentally benign alternative to conventional approaches. These organisms naturally generate metabolites known to serve as reducing and stabilising agents. The result being that the protocol is safe, inexpensive and simple to up-scale. New methods, including microwave-assisted synthesis, make processes even more efficient by enabling plant materials to heat very quickly via dielectric heating. Plant extracts are among the most attractive biological sources because they can produce nanoparticles at low concentrations without using hazardous chemicals [12-14].

Anethum graveolens, which belongs to the *Apiaceae* family, is a good example because it contains many flavonoids, polyphenols, antioxidants, vitamins, and minerals. Its aqueous extracts have antibacterial action against a wide range of pathogens, including Pseudomonas aeruginosa, *Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, and Shigella flexneri*. They may also lower cholesterol levels. In general, plant-based green synthesis, especially with *A. graveolens*, is a long-lasting and effective method for producing antimicrobial nanoparticles [15-18].

The novelty of the present work lies in three key aspects. First, while green-synthesized AgO and FeO nanoparticles have been individually reported in the literature, the specific binary AgO:FeO nanocomposite using *A. graveolens* extract as both reducing and capping agent has not been previously described. Most comparable studies have employed Ag/Fe_3O_4 or Ag/Fe_2O_3 systems using different plant sources or conventional chemical reducing agents. Second, the unique phytochemical profile of A. *graveolens* rich in flavonoids, polyphenols, and phenolic compounds confers distinct surface-capping chemistry that differentiates the resulting nanocomposite from previously reported systems. Third, unlike some previous studies in which composite formation did not significantly improve antimicrobial performance relative to individual nanoparticles, the synthesized AgO:FeO nanocomposite in this study clearly exhibited synergistic antibacterial activity, particularly against *S. aureus, S. epidermidis, E. coli*, and *Klebsiella sp*. Thus, the present study not only contributes to the green production and

characterization of AgO:FeO nanocomposites but also to the development of efficient antibacterial nanomaterials with broad-spectrum activity against clinically significant bacterial strains.

2. MATERIALS AND METHODS

It locally obtained plant of *Anethum graveolens* (Dill) Silver nitrate ($AgNO_3$) and pure ferric chloride ($FeCl_3$), was procured from a supplier located at 2048 E. Francis St., Ontario, CA 91761 USA.

2.1 Preparation of plant extract

In order to prepare 1 L of diluted dill extract, we added 5 g of plant powder in 50 ml deionized water and stirred it on a magnetic stirrer for 30 min. The solution was then filtered using filter paper. Figure 1 shows the plant extract of dill.

Figure 1: Aqueous extract of *Anethum graveolens* used in the green synthesis

2.2 Preparation of Nanomaterials

About 0.8494 g of pure $AgNO_3$ was dissolved in 100 ml of deionized water and stirred on a magnetic stirrer for two hours. During this process, the dill extract was gradually added dropwise to the $AgNO_3$ solution. A noticeable color change occurred, indicating the formation of nanosilver, as shown in Figure 2(a). A similar procedure was followed to prepare FeO nanoparticles. Briefly, 0.6338 g of $FeCl_3$ was dissolved in 100 ml of deionized water and stirred on a magnetic stirrer for 2 h. During this process, the plant extract was gradually added dropwise to the solution. A noticeable color change occurred, indicating the formation of iron nanoparticles, as shown in Figure 2(b).

(a) (b)

Figure 2: Visual observation of nanoparticle formation: (a) AgO and (b) FeO

The synthesis of the 0.05 M AgO:FeO nanocomposite was carried out in two sequential steps. First, AgO nanoparticles were green synthesized using *Anethum graveolens* extract as a reducing agent. 0.8494 g of silver nitrate were dissolved in 100 ml deionized water and stirred magnetically for 10 min to dissolve completely. We then combined 6 ml of the freshly prepared silver nitrate solution with A. graveolens extract in a proportion of 4 ml and maintained this mixture under magnetic stirring for 90 minutes. A distinct color change was observed in the reaction which confirmed the formation of AgO nanoparticlesthroughout the synthesis process. Step 2: Synthesis of AgO:FeO Nanocomposite FeO nanoparticles were prepared by dissolving 0.6338 g of pure ferric chloride in 100 ml of deionized water to obtain a 0.05 M solution. Under continuous magnetic stirring for 60 minutes this solution was applied as a gradual addition to the previously prepared AgO nanoparticle solution to promote nanocomposite mixed formation. The prepared AgO:FeO nanocomposite is illustrated in Figure 3.

Figure 3: Synthesized AgO:FeO nanocomposite via green method

2.3 Antimicrobial Test

The synthesized nanocomposite was evaluated for antimicrobial activity by agar well diffusion method. Tested strains were *S. aureus, S. epidermidis, E.coli and Klebsiella sp*., albicans. All assays were performed in triplets (n = 3) in wells of 6 mm diameter. A volume of ~1.5×10^8 CFU/ml (0.5 McFarland standard) was used to adjust bacterial suspension on the day of use. Plates were then incubated at 37 °C for 24 h.

3. RESULTS AND DISCUSSION

3.1 Structural Characterizations

The crystal structure and potential phase identity of the nanocomposite AgO:FeO produced through the green procedure were measured using XRD analysis (Figure 4). The observed diffraction peaks are both excellent in crystallinity and phase purity, with alleles matching (according to standard JCPDS reference files, including 41-1104 for AgO). So, XRD is an important technique in the identification of the crystalline structure and phase compositions, and estimation of crystallite size. XRD patterns confirm the formation of pure AgO, FeO, and AgO:FeO nanocomposites in a green synthesis context and demonstrate successful integration of the metal oxides within the composite matrix. Usually, distinct peaks with cubic or monoclinic AgO phase can be observed from the XRD pattern of AgO nanoparticles. The typical non-normalized characteristic peaks of FeO are located at the 2θ values related to hematite or magnetite structures according to the present phase (FeO, Fe_2O_3 and/or Fe_3O_4). These diffraction patterns verify the crystalline nature of FeO and aid in differentiating it from amorphous or impure forms. Especially for AgO:FeO nanocomposite.

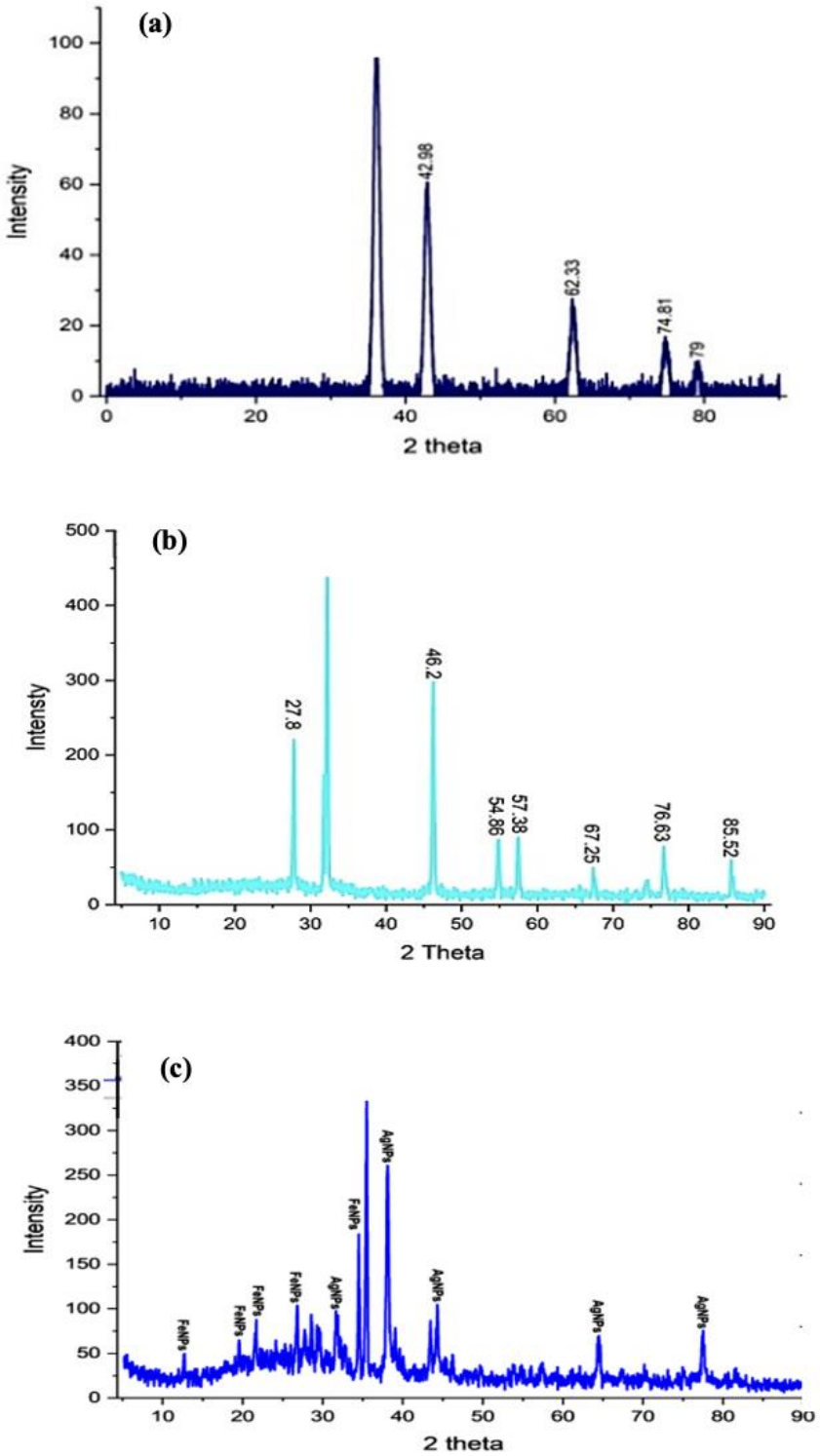

Figure 4: XRD patterns of (a) AgO, nano particles (b) FeO nano particles and (c) AgO:FeO nanocomposites

In the composite, broadening of these peaks may be indicative of smaller crystallites and larger surface area, which are two favorable characteristics for functional nanomaterials. Usually with the aid of the Debye–Scherrer equation, the crystallite size can be estimated and usually shows nanoscale dimensions. From the XRD analysis, crystalline AgO and FeO as well as their nanocomposite could successfully be made by following a green synthesis method producing pure, stable and well-structured metal oxide nanomaterials. The crystallite size was estimated using the Debye–Scherrer equation: $D = K\lambda/\beta\cos\theta$, where $K = 0.94$, $\lambda = 0.15406$ nm (Cu Kα radiation), β is the full width at half maximum (FWHM) of the diffraction peak, and θ is the Bragg angle. The mean crystallite sizes were calculated to be 22.4 nm for AgO, 18.7 nm for FeO and 20.1 nm for the AgO:FeO nanocomposite (confirming their nanoscale nature).

The small decrease in crystallite size of the composite compared to pure AgO is consistent with structural distortion due to the incorporation of two metal oxide phases. The typical diffraction peaks were consistent with FeO and AgO nanoparticles, thus confirming the successful synthesis of these components in both forms as well as their nanocomposite. The FeO nanoparticles diffractions peaks at the 2θ values of 36.11 °, 42.98 °, 62.33 °, 74.81 ° and 79 ° correspond to the characteristic planes of iron oxide nanostructures assignments. These results are consistent with earlier studies which reported diffraction reflections at approximately 35–36 °, 43 °, 57–63 °, and 74–79 ° corresponding to crystalline iron oxide nanoparticles of cubic spinel structures [19,20]. The well-focalised diffraction peaks suggest the formation of highly crystallized, nanoscale FeO particles. In addition, there is no evidence of secondary diffraction peaks demonstrated that the nanoparticles prepared were highly phase pure. The AgO nanoparticles also showed diffraction peaks at 27.8 °, 32.32 °, 46.2 °, 54.86 °, 57.38 °, 67.25 °, 76.63 °, and 85.52 °. It indicates that the silver oxide nanoparticles possess a crystalline structure. The diffraction peaks match previous studies of AgO and Ag_2O nanostructures which showed that the unique reflections at 27 °, 32 °, 46 °, 54–57 °, 67 ° and 76 ° were characteristic of crystalline silver oxide phases [21,22]. The observed peaks confirm that silver was oxidised and yield well-crystallised AgO nano particles.

Similarly, the lack of impurity peaks suggests that the produced silver oxide nanoparticles are high purity and structurally stable. The XRD diffractogram of the AgO:FeO nanocomposite confirmed that the hybrid nanocomposite was successfully made, as it showed the typical diffraction peaks of both AgO and FeO nanoparticles. The presence of AgO and FeO diffraction peaks that co-exist without additional impurity phases confirms that both metal oxides remained crystalline in the final material produced. Similar results for Ag/Fe-based nanocomposites, where diffraction peaks corresponding to iron oxide and silver phases were revealed. This demonstrated that the two nanomaterials had hybridised and interacted in their interfaces. In addition, the maintenance of the different diffraction peaks in AgO:FeO nanocomposite indicates that both components are structurally compatible and stable with each other which reflects the enhancement of their physicochemical, catalytic and antibacterial properties when compared to individual nanoparticles [23].

The FESEM analysis has also confirmed both phase and crystal structure, in given via above mentioned XRD (Figure 5). The morphological features of the synthesized nanoparticles are clearly depicted in (Figure 5(a) and Figure 5(b)), this will depend on which additional salt is used in the previously described method, in this case AgO with quasi-spherical shapes agglomerated by high surface energy at nanoscale (Figure 5(a)). In contrast, FeO nanoparticles exhibited an irregular cluster morphology (Figure 5(b)), indicating variable aggregation and a range of growth behavior during synthesis. In addition, the particle size distribution of the AgO:FeO nanocomposites illustrated in Figure 5(c) revealed a relatively wide-size distribution with an average diameter pair (163.7 ± 48.34 nm) The increasing of Size Distribution is due to nanoparticle agglomeration caused by interparticle forces and surface-to-volume ratio. These observations themselves confirmed that the green route synthesis generates aggregated nanostructures with a range of different shapes and sizes.

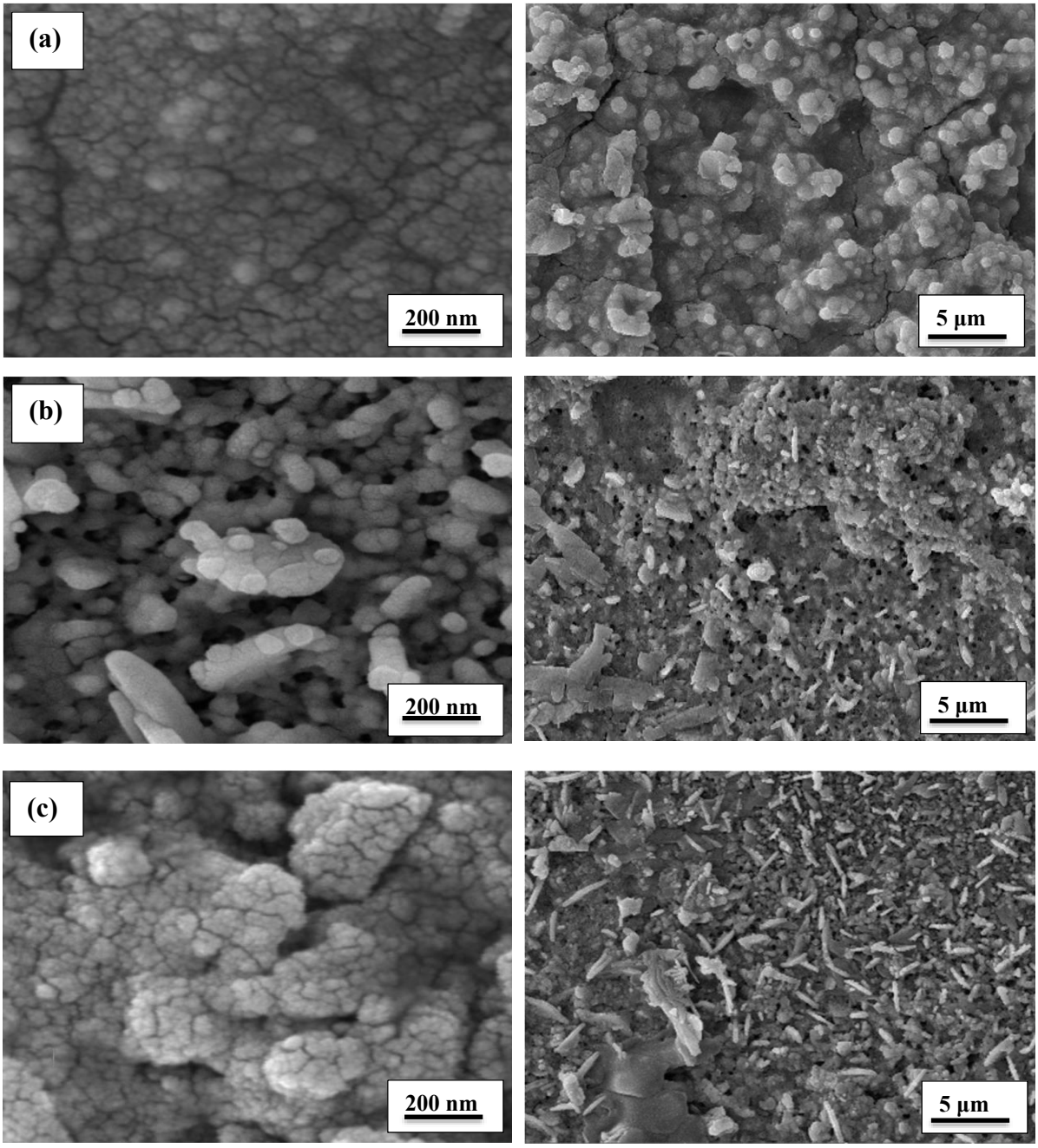

Figure 5: FESEM images of (a) AgO, (b) FeO, and (c) particle size distribution of AgO:FeO nanocomposites

The elemental characterization of the manufactured AgO:FeO nanocomposites are shown in Figure 6. Figure 6(a) presents the EDX data, which confirms that silver (Ag), iron (Fe) and oxygen (O) are positively combined in order to obtain the nanocomposite with no undesired impurities. The related FE-SEM image of the area under analysis is shown in Figure 6(b), it presents the sample surface morphology which was targeted for element identification. In addition, the elemental mapping in Figure 6(c) shows a more uniform spatial distribution of Ag, Fe and O throughout the nanocomposite which indicates complete mixing of all elements at nanoscale.

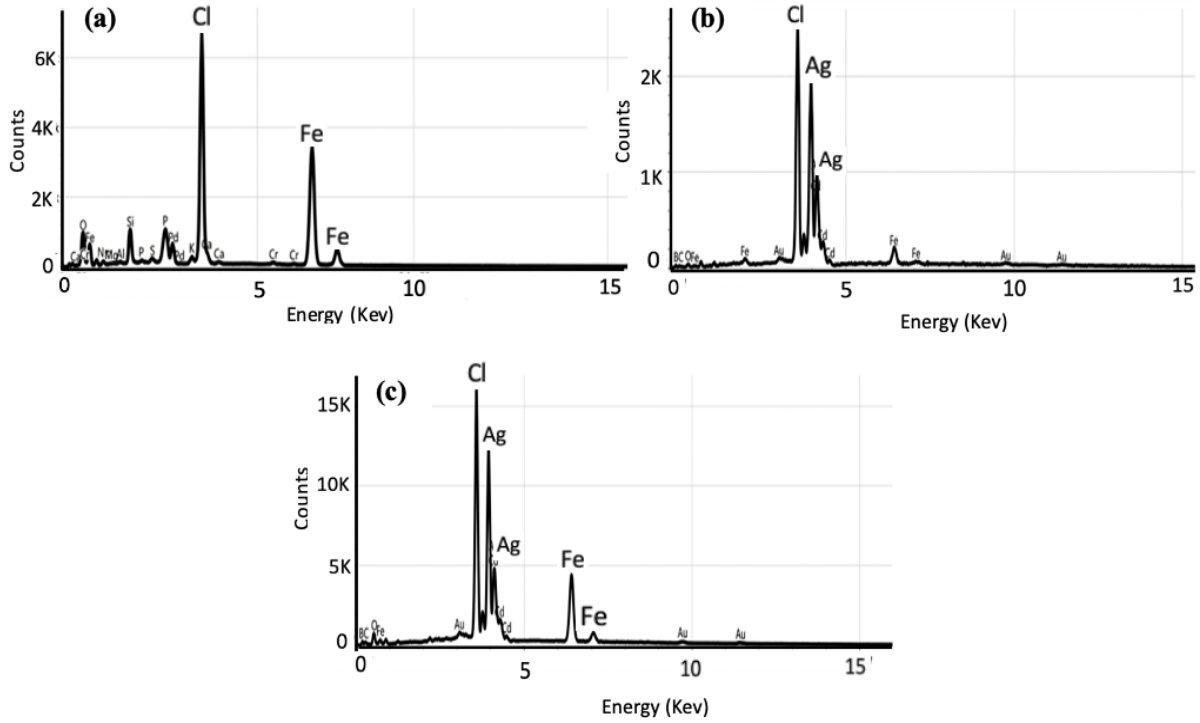

Figure 6: EDX spectrum of (a) FeO, (b) AgO, and (c) AgO:FeO nanocomposites

The active principles in *Anethum graveolens* leaf extract acted by decreasing some Ag^{+} ions, promoting the formation of Ag NPs and simultaneously stabilizing them via capping. FTIR spectroscopy was performed to identify the biomolecules responsible for these effects, as demonstrated in Figure 7. FTIR spectroscopy is an important analytical technique to determine the functional groups and for verification of metal oxide nanoparticles and their composites. FTIR analysis helps in understanding the chemical bonds as well as interactions present during nanoparticle formation, especially in the case of green synthesis including plant extract or any eco-friendly reducing agents.

In general, FTIR spectrum of AgO nanoparticles shows also the peaks for itself in the range of 400–600 cm^{-1} due to stretching vibrations of AgO. The broad absorption bands that are found from 3200–3500 cm^{-1}, and at around1600 cm^{-1} stems from the –OH stretching and bending vibrations (which may be due to water molecules or phytochemicals existing in plant extracts). In the same fashion, a characteristic absorption band in 500–600 cm^{-1} region represents Fe–O stretching vibrations for FeO nanoparticles. Similar FTIR assignments are reported for the phytochemicals coated iron oxide nanoparticles based on recent review in Nanoscale Advances (2026). It showed that there were broad O–H bands in 3200–3500 cm^{-1} and carbonyl-related peaks in the range of 1600–1700 cm^{-1}, which were related to plant metabolites helping stabilize nanoparticles [24].

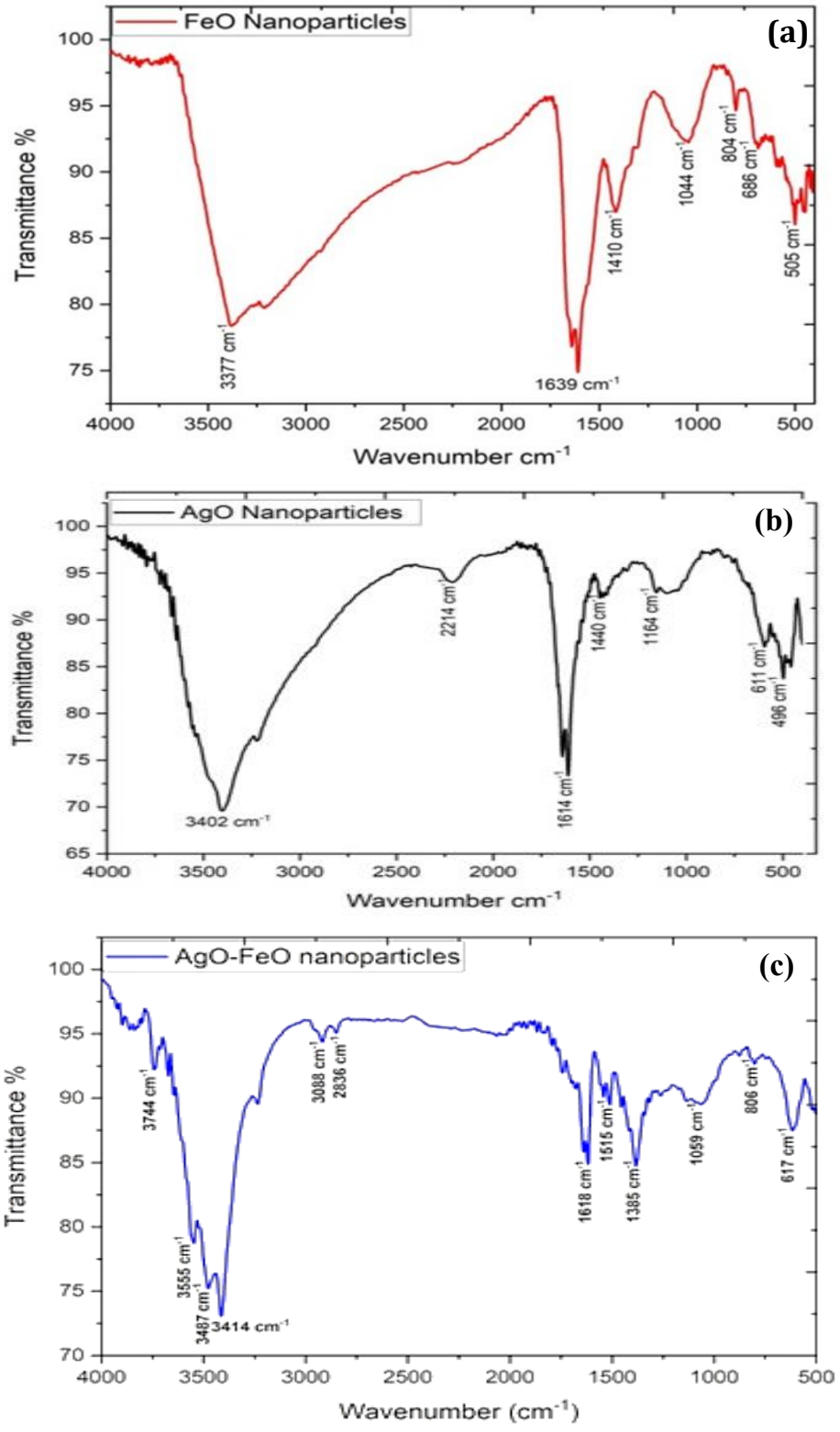

Figure 7: FTIR spectra of AgO:FeO nanocomposites synthesized via green method

In addition, other peaks associated with organic remains, e.g. flavonoids and phenolic compounds from the extract confirm more evidence related to bio-organic-molecules stabilizing or capping the nanoparticles. The FTIR spectrum of the AgO:FeO nanocomposite shows overlapping signals of Ag–O and Fe–O vibration bands, thereby confirming the coexistence of two metal oxides. More commonly broader peaks associated with stretching of OH and C=O may indicate the presence

of a stronger interaction between these metal oxides and the phytochemicals occurring in the green synthesis. In second the work of biogenenic answill elucidated in research done by Iheme et al. [25] then with Ag–O vibrations observed at position 467 cm^{-1} and Fe–O vibrations was around 593 cm^{-1} for biosynthesised Ag–Fe nanocomposites. The scientists additionally demonstrated that the natural functional groups from the plant extracts were important for coagulating nanoparticles and adding additional functionality on their surfaces.

In general, the FTIR study supports that AgO, FeO and their nanocomposite had formed. So it indicates for bioactive functional groups that involved during the reduction, stabilization, and capping processes confirming green synthesis as eco-friendly/biocompatible synthesized nanomaterials.

3.2 Optical Properties via UV-Vis Spectroscopy

The UV-Vis absorption spectroscopy technique covers the wavelength range of 200–1100 nm, which is widely used to explore the optical properties and electronic structure of nanocomposites. Synthesis of metal oxide nanoparticles via green methods, characterization and their photoluminescence properties via UV visible spectroscopy; this assay is an important tool for elucidation. UV-Vis spectroscopy shows characteristics of parameters including the band gap energy, light absorption properties and its possible application as photocatalyst, sensing and optoelectronics.

The different absorption behaviors in case of AgO, FeO and AgO:FeO composites were observed in their UV-Vis spectra as shown in Figure 8 (a-c). The strong absorption peak in the ultraviolet region is remarkable and the resonance assigned by electrodynamics theory known as surface plasmon resonance (SPR), which refers to a coherent oscillation of conduction electrons at a metallic surface created by incident photons in silver nanoparticles. The exact position and intensity of this absorption peak is largely dependent on nanoparticles size, shape and surrounding medium. These spectral characteristics reinforce the successful preparation of nanomaterials and impart useful information about their possible functional functions.

The optical band gap energies of the synthesized nanomaterials were evaluated following Tauc plot using the relation: $(\alpha h\nu)^2 = A(h\nu - Eg)$ where α is absorption coefficient, $h\nu$ is photon energy and Eg is its optical band gap. These were found to be approx 1.82 eV for AgO, 2.14 eV for FeO and approximately 1.95 eV for the respective nanocomposite of AgO:FeO, respectively. The intermediate band gap of the nanocomposite (LZO1) between its constituent phases, as well as charge transfer at this interface, implies electronic interaction that may lead to improved photocatalytic and antimicrobial performance.

Similar behavior was found for surface plasmon resonance of optical absorption in gold nanoparticles due to particle size and morphology [26]. Silver nanoparticles have been shown to not only improve optical and electronic properties but also serve as an aid to improve charge transfer behavior within photodetector-based systems [27].

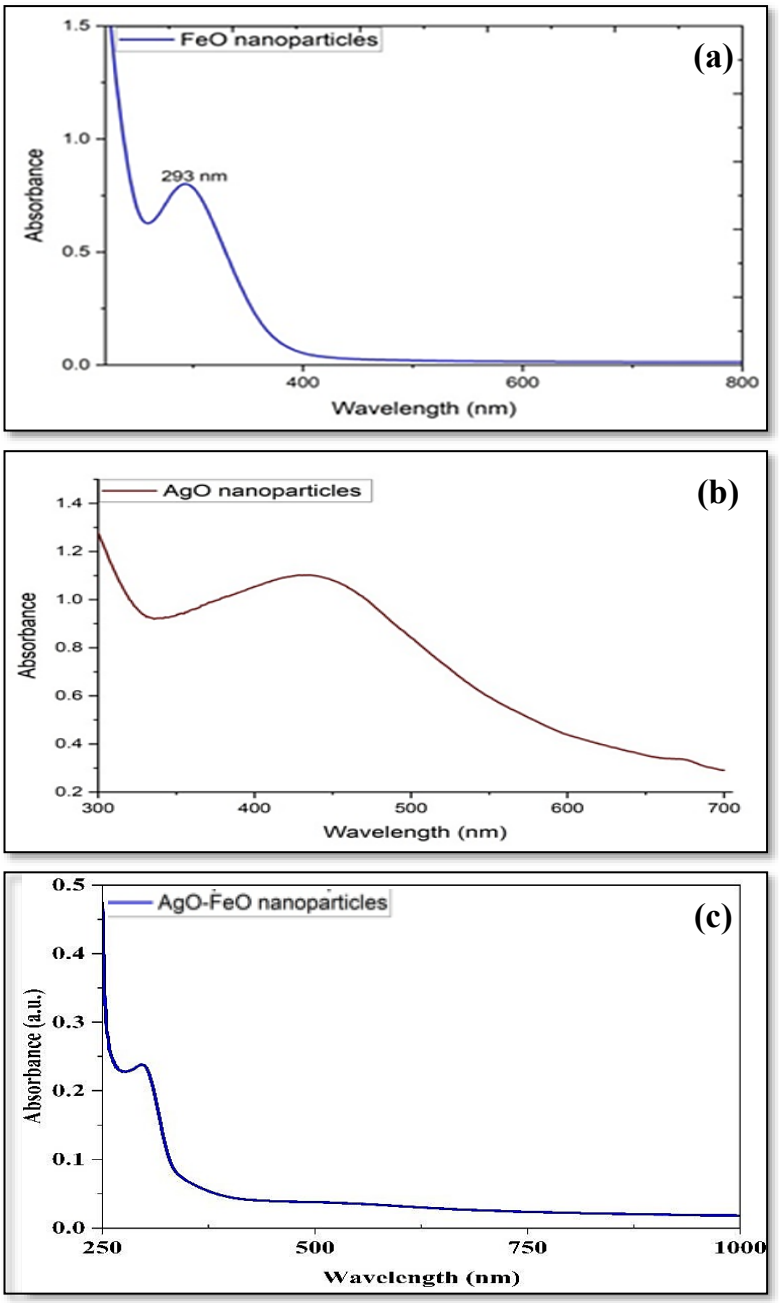

Figure 8: UV–Vis absorption spectra of (a) AgO, (b) FeO, and (C) AgO:FeO nanocomposites

3.3 Biological Analysis

Antimicrobial effectivity test on biosynthesized AgO:FeO nanocomposits was executed on a group of model organisms such as *S. aureus, S. epidermidis, E. coli and Klebsiella sp*. Figure 9 and Table 1 show the antimicrobial activity results (all microplate wells containing trypan blue) for 60C38650 with nanocomposites from both bacteria (Gram−positive and Gram−negative) & fungal strains. With the combined characteristics of two or more metal-based nanoparticles, studies on antibacterial properties and mechanisms have recently been conducted for hybrid nanocomposites with high biological activity. As an example, the coupling of iron oxides with graphene-carbon nanotubes has demonstrated a powerful water disinfection. Such enhancement antibacterial effectiveness can be correlated with the large surface area of composite materials leading to better adsorption capacity as compared to conventional adsorbents. In accordance with these findings, our study proposes that the

implementation of AgO and FeO into one nanocomposite works to improve antibacterial performance because of the synergistic effects of each metallic component [28].

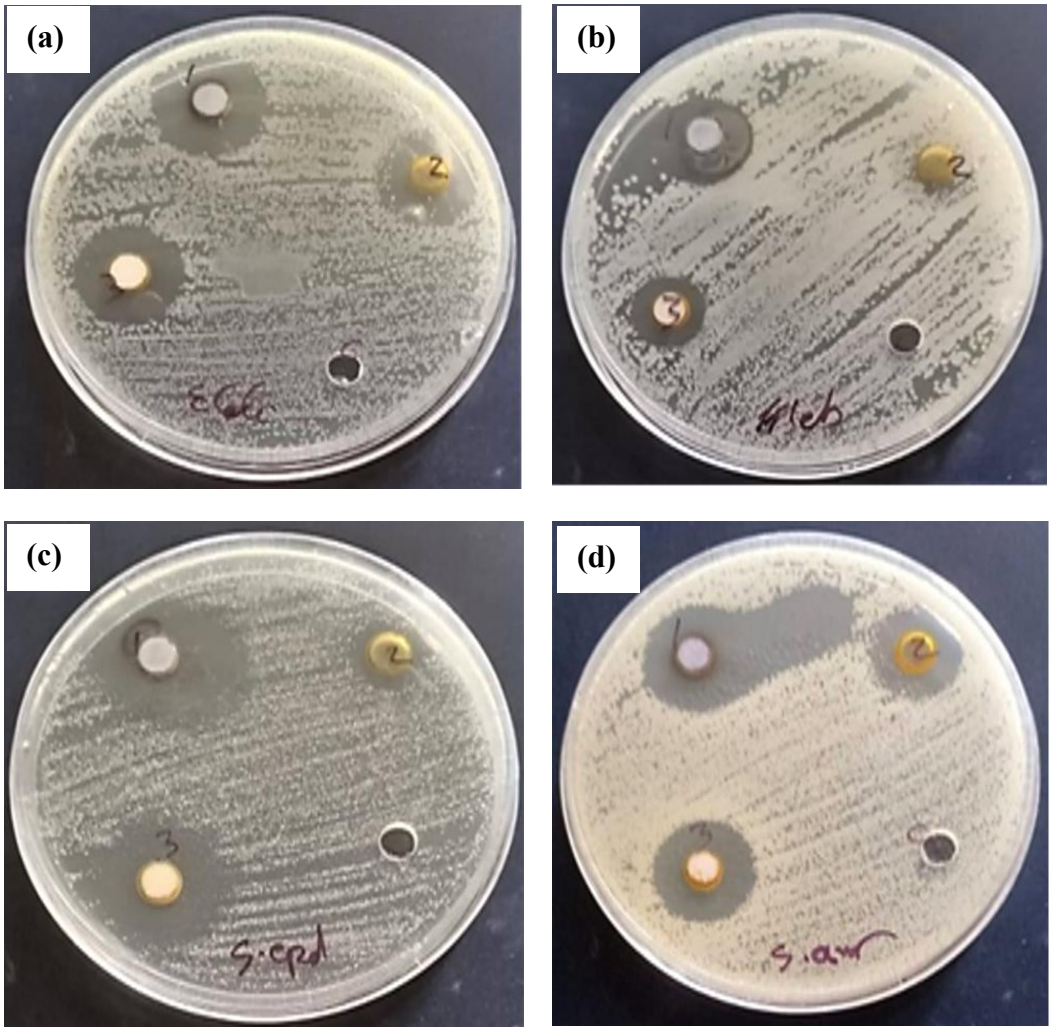

Figure 9: Antimicrobial activity of AgO:Fe on the different concentrations for some selected bacterial strains (a) *E coli*, (b) *klebsiella sp* (c) *S. epidermidis* and (d) *S. aureus*. The resultant data shows a dose-dependent inhibition, which illustrates the nanocomposites are antimicrobial agents

Attachment of nanoparticles to the bacterial cell membrane is the first step in their antibacterial action. However, the exact mechanism of nanoparticle binding has not been fully elucidated, but it is generally accepted that electrostatic binding is crucial to the adhesion between bacterial membranes and particles. Upon approach to the membrane, FeO nanoparticles facilitate reactive oxygen species (ROS) production that induces oxidative stress resulting in "pitting" of the membrane and ultimately cell death. Physical properties play an important role in antibacterial activity, particle size, surface area and crystallinity. In particular, reduced crystallinity and particle size as well as an increased surface area have been found to be associated with improved ROS generation and more potent antimicrobial properties. These characteristics have shown more powerful antimicrobial activity in nanostructures like smaller ZnO nanoparticles, for example.

The naturally large surface area of FeO provides an excellent platform for substantial ROS production, which in turn causes significant membrane damage to bacteria. Higher crystallinity, on the

other hand, might limit this effect by lowering ROS generation and nanoparticle ion release, thus resulting in reduced antibacterial activity [29]. In the current study direct experimental evidence is lacking for ROS generation.; however, the observed concentration-dependent inhibition zones and the synergistic activity of the AgO:FeO nanocomposite where the composite (23.67 mm against S. epidermidis) outperformed individual AgO (21.33 mm) and FeO (15.67 mm) components are experimentally supported findings that align with ROS-based synergism reported in similar bimetallic nanocomposite systems. Direct quantification of ROS (e.g. DCFH-DA fluorescence assay) will be needed in the future to experimentally confirm this mechanism-driven interpretation. When compared to routine clinical antibiotics such as gentamicin, ciprofloxacin and ampicillin that typically yield inhibition zones (ZOI) of 15-25 mm, the antimicrobial capacity of AgO NPs, FeO NPs and AgO: FeO nanocomposites (NCs) illustrates a high-scale possibility for therapeutic response [30]. The AgO: FeO NC was the best system, with an ideal ZOI range of 16.00 ± 1.00 mm to 23.67 ± 0.58 mm, whereas AgO NPs showed excellent potency (21.33 ± 1.00 mm to 22.00 ± 1.00 mm) and FeO NPs showed very moderate solo activity (0.00 mm to 19.00 ± 1.00 mm).

Notably, the nanocomposite successfully closed the resistance gap that iron oxide alone could not overcome by outperforming pure silver against *S. epidermidis* and regaining activity against *Klebsiella sp*. This improved performance, which mimics or surpasses the performance of conventional drugs while providing a lower silver load for improved biocompatibility, is attributed to a synergistic multi-target mechanism that includes sustained Ag^+ ion release, enhanced Reactive Oxygen Species (ROS) generation via the FeO matrix, and physical membrane disruption. These results place these nanocomposites as reliable, broad-spectrum substitutes for conventional pharmaceutical agents in biomedical applications and are consistent with recent research highlighting the role of bimetallic synergies in overcoming antimicrobial resistance [30,31].

Table 1: Inhibition zones (mm) of AgO, FeO, and AgO:FeO nanocomposites at a fixed concentration against selected microbial strains

Bacteria	Inhibition zone (mm)		
	AgO NPs	FeO NPs	AgO:FeO NCs
E. coli	22.00±1.00	19.00±1.00	20.00±0.00
Klebsiella sp.	16.00±0.00	0.00±0.00	16.00±1.00
S. aureus	21.33.00±1.16	18.67±0.58	19.00±0.00
S. epidermidis	21.33±1.15	15.67±0.58	23.67±0.58

Ag NPs, on the other hand, exert their antibacterial effects through sustained release of silver ions upon contact with bacterial membranes. This causes membrane disruption, higher permeability and structural disturbance of important transport systems in cells. Theoretical models suggested that Ag NPs interact with bacterial membranes, which could be mediated by uncharged polymer molecules or other mechanisms that go beyond the context of simple electrostatic binding. The damage results in the formation of pits, leak of intracellular content such as proteins and lipopolysaccharides and finally cell lysis. This "pitting" occurs more readily in Gram-negative bacteria such as *E. coli,* and the thick peptidoglycan layer found in Gram-positive bacteria like *S. aureus* may blunt this effect. Nevertheless, Ag NPs can still upset homeostasis through targeting of Gram-positive bacteria metabolism and its cell wall biosynthesis. Because of structural differences in their cell walls, Gram-positive strains generally require higher nanoparticle concentrations for inhibition than Gram-negative ones. Nevertheless, this generalization does not appear to hold for the results obtained in our study, as AgO:FeO nanocomposite demonstrated high antibacterial activity on both bacterial types. This unusual phenomenon was possibly due to the unique Ag–O–Fe interaction caused by the synergetic effect of AgO and FeO in the nanocomposites, leading to better physicochemical properties and wider antimicrobial activity [32].

4. CONCLUSIONS

This study successfully developed eco-friendly synthesized AgO:FeO nanocomposites using *Anethum graveolens* plant extract as a natural reducing and stabilizing agent. The successful formation and incorporation of silver oxide and iron oxide phases within the nanocomposite were confirmed using comprehensive characterization techniques, including XRD, SEM, EDX, FTIR, and UV-Vis spectroscopy. The scanning electron micrograph (SEM) analysis showed that the nanoparticles were around 164 nm in size and exhibited moderate agglomeration, which may affect surface-related properties. Biological evaluation showed that the AgO:FeO nanocomposite displayed remarkable antimicrobial potential towards wide range of pathogens including Gram-positive (e.g., *S. aureus and S. epidermidis*) as well as pathogenic Gram-negative species (e.g., *E. coli and Klebsiella sp*.) micro-organisms. The results suggest a synergistic antibacterial effect of the nanocomposite that does not lead to reduced effectiveness compared to its single components probably due to enhanced ROS generation and increased interaction surface with microbial cells. The results of this study indicate that green-synthesized AgO:FeO nanocomposites may serve as effective, eco-friendly antimicrobial agents in biomedical and environmental applications. Much effort is still needed to prove the synthetic cytotoxicity, mechanistic pathways as well as long-term stability toward practical applications. Because of its medical nature, biocompatibility still plays an important role. Although cytotoxicity testing was not conducted in this study, green-synthesized nanoparticles bonded with polyphenols of *A. graveolens* particularly flavonoids are typically known for lower cytotoxic profiles when compared to chemically synthesized counterparts. This study highlights a formal biocompatibility assessment (MTT cytotoxicity assays on mammalian cell lines followed by hemocompatibility testing) as an important next step before any biomedical application. Future work should also include determination of MIC and MBC values to allow for a more rigorous, clinically useful quantification of antimicrobial potency.

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Author Contributions

Mohammed Zorah designed/conceived the work, carried out experimental work and wrote the manuscript. Hasan Bakheet Jasim provided input into sample preparation and laboratory investigations, along with support for reagents and research materials. Hayder Ayyed Naser carried out data collection and experimental validation and provided assistance for the analytical measurements. Mustafa Mudhafar conducted data analysis and interpretation and contributed to the revision of the manuscript. Che Nor Aiza Jaafar supervised the research methodology and critically reviewed the manuscript for intellectual content. Ismail Zainol provided technical guidance and project administration and finalized the manuscript for publication.

Disclosure of Conflict of Interest

The authors declare no competing interests.

Compliance with Ethical Standards

This work is compliant with ethical standards.

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