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Research Article

Antibacterial Efficacy of Phytosynthesized Multi-Metal Oxide Nanoparticles against Drug-Resistant Foodborne Pathogens

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Human health is threatened worldwide by microbial infections. Antibiotic overuse and misuse have resulted in antimicrobial-resistant bacteria. To battle such resistant microbes, we are looking for safe and alternative antimicrobial treatments, and the advent of nanotechnology holds promise in this regard. Metal oxide nanoparticles have emerged as a promising alternative source for combating bacteria resistant to various antibiotics over the last two decades. Due to their diverse physicochemical characteristics, metal oxide nanoparticles can operate as antibacterial agents through various methods. In the present research, six types of metal oxide NPs were synthesized and characterized (XRD, FTIR, SEM with EDAX, and TEM) from different plants such as *Hydrangea paniculata* (for NiO NP synthesis), *Plectranthus amboinicus* (for ZnO-NP synthesis), and *Andrographis paniculata* (V_2O_5 NPs). On the other hand, drug-resistant pathogens were isolated from clinical samples, those who suffered from foodborne illness. V_2O_5 NPs produced from *Andrographis paniculata* plant extract have much higher bactericidal efficacy than other metal oxide NPs against all three bacterial strains. Sensitive bacteria included *S. aureus* and *E. coli*, followed by *K. pneumoniae*. As a result, structural characterization was used to further screen V_2O_5 NPs. The orthorhombic structure of the crystallites was confirmed by XRD, with an average crystallite size of 20 nm. The absorbance spectrum and functional groups were identified using UV-visible spectral analysis and FTIR. SEM and EDX identified spherical-shaped NPs, and particle size (58 nm) was confirmed by transmission electron microscopy (TEM). As a result, we hypothesized that bioinspired V_2O_5-NPs could be employed as a possible antibacterial agent against drug-resistant pathogenic bacteria to replace currently existing inefficient antibacterial drugs.

1. Introduction

Foodborne infectious diseases caused by microbial pathogens are considered as a serious issue in developed as well as developing countries [1]. Diseases like hepatitis, typhoid, and cholera are often caused owing to the contamination in food materials by microbes such as *E.coli*, *Salmonella*, and *Shigella* and are easily transmitted through the unhygienic handling of food, contaminated water, and contact with animals [2]. Several researchers reported the emerging of pathogens resistant to currently available antimicrobial agents as a serious effect on human health [3]. Antibiotic resistance has reached epidemic proportions in the previous decade, according to the World Health Organization, posing a severe threat to world health [4]. Each year, antibiotic-resistant bacteria kill about 700,000 people worldwide [5]. Researchers from the public, private, academic, and food industries are all working hard to combat the growing epidemic of drug-resistant diseases [6]. As a result, progress is contingent on well-coordinated activities across sectors to address cross-cutting concerns in animal and human health, agriculture, food, and the environment [5]. The drug-resistant pathogens are developed by the changes in gene expression by environmental stress and overuse or misuse of antibiotics [7, 8]. In this scenario, potential antibacterial agents are highly required to treat infections caused by drug-resistant pathogens. Other than the medical field, antibacterial agents are crucially playing a role in the textile industry, paint industries, water purification system, food packaging, and preservation field [9].

Nanotechnology is booming as a fascinating branch of science and produces nanoscale (1-100 nm) materials for broad applications [10]. In science and technology, metal oxide NPs play a crucial role in many applications. In medical applications, the usage of nanomaterials and metal oxide NPs is rapidly mounting in cancer treatment, antimicrobial therapeutic agent, biosensing, chemotherapy, and imaging purposes due to its specific applications like surface to volume, size, and morphological features [11–13]. Various physical and chemical methods are adopted for the synthesis of metal oxide NPs [14]. Physical methods require more energy, and chemical approaches utilize a range of chemicals as a precursor and reducing agents that produce toxic by-products during the synthesis of nanoparticles, and yield percentage is also low [15, 16]. To overcome the limitations of conventional methods, green synthetic technologies are gaining a lot of traction in current materials science development and study. Green nanoparticle synthesis, as created by regulation, clean-up, and control and remediation methods, will primarily improve their eco-friendliness. As a result, several components such as pollution reduction, nontoxic solvent use, waste prevention, and renewable feedstock can be used to characterize certain basic principles of biosynthesis [17]. Biosynthesis is required to avoid the development of toxic by-products in a sustainable and environmentally responsible manner. Several biological entities, such as plant extracts, bacteria, and algae, have been accommodated by biosynthesis of metal and metal oxide nanoparticles. Using the plant is a quick, easy, and simple way to synthesis metal and metal oxide nanoparticles among the available green ways to nanoparticle creation [18].

The diverse group of researchers reported the activity of green synthesized metal oxide NPs against drug-resistant pathogens. The metal oxide NPs developed by green route which include ZnO [19], CuO [20], NiO [21], MoO_3 [22], and V_2O_5 [23] showed efficient bactericidal activity against an extensive range of drug-resistant bacteria such as *E. coli*, *S. aureus*, *K. pneumoniae*, *B. cereus*, *L. monocytogenes*, and *S. typhi*. Gram-positive and Gram-negative bacteria, as well as spores resistant to high temperature and high pressure, were all killed by ZnO nanoparticles [24]. CuO nanoparticles demonstrated substantial antibacterial activity against a variety of bacterial strains (*E. coli*, *P. aeruginosa*, *K. pneumoniae*, *Enterococcus faecalis*, *Shigella flexneri*, *S. typhimurium*, *Proteus vulgaris*, and *S. aureus*) [25]. *E. coli* and *E. faecalis* were the pathogens with the highest sensitivity to CuO nanoparticles. The most vulnerable strains to NiO nanoparticles were *Bacillus licheniformis* and *Bacillus subtilis*, while *Klebsiella pneumoniae* was the least susceptible strain in the zone of inhibition [26]. V_2O_5 nanoparticles had good antibacterial efficacy against pathogens including *Escherichia coli* and *Staphylococcus aureus* [27]. An outstanding antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus flavus*, and *Candida albicans* was shown by MoO_3 nanoparticles [28].

Moreover, Hajipour et al. [29] reported the reusability of metal oxide NPs as an antibacterial agent. The present evaluation is aimed at exposing the antibacterial potency multitype metal oxide NPs synthesized by plant extracts against drug-resistant pathogens. In this present study, the metal oxide NPs with higher potency have been selected for further characterization studies.

2. Materials and Methods

2.1. Isolation and Identification of ESBL Bacterial Strains. The drug-resistant pathogens were isolated from clinical samples (stool) collected from patients infected with foodborne illness. The ESBL strains were screened based on the antibiotic sensitivity test and double-disk potentiation procedures suggested by the National Committee for Clinical Laboratory Standards (NCCLS) using two types of drugs, ceftazidime (30 μg) and ceftazidime+clavulanic acid (30 μg/10 μg). The isolated drug-resistant strains were cultured in nutrient agar medium composed of peptone (10 g), beef extract (1 g), sodium chloride (5 g), agar (10 g), and double-distilled (DD) water (1000 mL).

2.2. Phytosynthesis of Metal Oxide Nanoparticles

2.2.1. Preparation of Plant Extracts. The metal oxide NPs were synthesized using aqueous extracts obtained from three types of plants such as *Hydrangea paniculata* (for NiO NP synthesis), *Plectranthus amboinicus* (for ZnO-NP synthesis), and *Andrographis paniculata* collected from local areas of Tiruchirappalli District, Tamil Nadu. For the preparation of CuO, V_2O_5, and MoO_3 NPs, leaves of *A. paniculata* were cleaned thoroughly by distilled water and dried at 303 K.

Table 1: Bactericidal activity of metal oxide NPs against *S. aureus*.

Nanoparticles	Zone formation (mm) at different concentrations			
	Control (d.H_2O)	25 mg/50 μL	25 mg/100 μL	50 mg/50 μL
MoO_3	—	—	—	—
CuO	—	—	—	18 ± 1.41
NiO	—	7 ± 2.8	9 ± 1.09	18 ± 2.1
ZnO	—	—	—	—
V_2O_5	—	11 ± 1.4	16 ± 0.7	17 ± 0.4

Dried leaves were weighed (5 g), immersed in 100 mL of DD water, and boiled at 353 K for 1 h to obtain the extract. The extract was filtered twice to eliminate the residual solids. The green-colored boiled extract (reducing agent) was used for the synthesis of NPs.

2.2.2. Synthesis of NiO NPs. The nickel nitrate (1 mM) and *Hydrangea paniculata* flower extracts were used (1 : 1 *v*/*v*) and kept under the dark condition for 12 to 24 hrs of the incubation period. The resulting precipitate was centrifuged for 30 minutes at 6500 rpm, washed with double-distilled water to eliminate contaminants, and dried at 60°C for 6 hours. The powder that resulted was employed in subsequent research.

2.2.3. Synthesis of ZnO-NPs. In this protocol, 2 mL of *Plectranthus amboinicus* plant extract was added dropwise to 100 mL of zinc oxide (0.1%) solution (thoroughly mixed for 10 mins by magnetic stirrer). The ZnO-NPs were formed as white crystalline, which was washed several times with water, filtered, and dried at 333 K.

2.2.4. Synthesis of CuO, V_2O_5, and MoO_3 NPs. To synthesize, these metal oxide NPs, stoichiometric amounts of Cu $(CH_3COO)_2{\cdot}H_2O$ and ammonium metavanadate, and chloroethoxide ($MoCl_5$) were mixed with 30 mL DD water and mixed with 20 mL of *A. paniculata* plant extract without foam. Unreacted compounds were removed using DD water. The end products were dried at 453 K for 20 mins and calcinated at 673 K for 2 hrs. The obtained powders were used for the evaluation of bactericidal activity.

2.3. Assay of Bactericidal Activity of Green Synthesized Metal NPs. Metal oxide NPs were screened for their antibacterial activity against three drug-resistant foodborne pathogens *E.coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* by well diffusion method. The pH of Muller Hinton agar media was adjusted to 7.3 ± 0.2 at 25°C. Using gel puncture, form 6 mm diameter of well on the media. Using a sterile cotton swab, the bacterial cultures were spread all over the media separately. The concentration ranges from 25 mg/50 μL, 25 mg/100 μL, to 50 mg/50 μL of water as control, liquid culture filtrate, and CuO, NiO, ZnO, MoO_3, and V_2O_5 were loaded into the well using a micropipette and then incubated at 35°C for 18 hrs, and inhibition zones were measured for different concentrations.

2.4. Structural Characterization of V_2O_5 Nanoparticles. The crystalline nature, functional groups, and morphological with elemental composition features were analyzed by XRD, FTIR, SEM with EDAX, and TEM analysis.

3. Results and Discussion

For the assessment of the antibacterial efficacy of metal oxide NPs, β-lactamase-producing strains were isolated from the stool samples of diverse age groups of patients. 876 β-lactamase-producing bacterial strains were isolated for a period of 3 months from March 2019 to May 2019 [30]. The ESBL-producing *E.coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* (MRSA) were screened and isolated based on the production of β-lactamases and picked for biocidal analysis. The inhibition zone of combination disk of ceftazidime+clavulanic acid was ≥5 mm, when compared to ceftazidime disk alone, which confirmed the ESBL production [30].

3.1. Bactericidal Properties of Metal Oxide Nanoparticles. The bactericidal activity of green synthesized metal oxide NPs such as NiO, ZnO, CuO, MoO_3, and V_2O_5 was tested by the well diffusion method against three drug-resistant strains (*Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*) (Tables 1, 2, and 3; Figures 1–3). Among the NPs, V_2O_5-NP showed effective resistance against all three strains. NiO NP (50 mg/50 μL) was found to be the most effective and exhibited maximum zone of inhibition against *S. aureus*. Seven and 9 mm sized inhibitory zones at 25 mg/50 μL and 25 mg/100 μL of concentration against *S. aureus*. But, no zone was found against the other two strains. Srihasam et al. [31] have described the antibacterial efficiency of NiO NPs synthesized using Stevia leaf extract as reducing agent and resulted inhibition zone against *E. coli* (16 mm), *B. subtilis* (15 mm), and *S. pneumoniae* (14 mm). Likewise, Abbasi *et al.* [32] also have reported the bactericidal potential of *Geranium wallichianum* plant-mediated NiO NPs against *E. coli* and *S. aureus*. Maximum inhibition zone was observed in the Gram-negative strains due to the easy penetration of NiO NPs to the cell membrane with the lack of (lipopolysaccharide) peptidoglycan layer [33]. There was no inhibition zone observed in the plates introduced with MoO_3–NP P.

CuO-NP explored its bactericidal action in contradiction of *E. coli* and *S. aureus* with 18 mm diameter sized inhibitory zone at the concentration 50 mg/50 μL. No zone was observed against *K. pneumoniae* which indicates its resistant activity against CuO NPs. Ahamed et al. [25] have reported the antibacterial activity of CuO NPs against various

Table 2: Bactericidal activity of metal oxide NPs against *E.coli*.

Nanoparticles	Zone formation (mm) at different concentrations			
	Control	25 mg/50 μL	25 mg/100 μL	50 mg/50 μL
MoO_3	—	—	—	—
CuO	—	—	—	18 ± 2.12
NiO	—	—	—	—
ZnO	—	—	—	—
V_2O_5	—	8 ± 0.7	12 ± 1.06	14 ± 1.7

Table 3: Bactericidal activity of metal oxide NPs against *K. pneumoniae*.

Nanoparticles	Zone formation (mm) at different concentrations			
	Control	25 mg/50 μL	25 mg/100 μL	50 mg/50 μL
MoO_3	—	—	—	—
CuO	—	—	—	—
NiO	—	—	—	—
ZnO	—	—	—	—
V_2O_5	—	12 ± 1.04	13 ± 1.7	14 ± 1.76

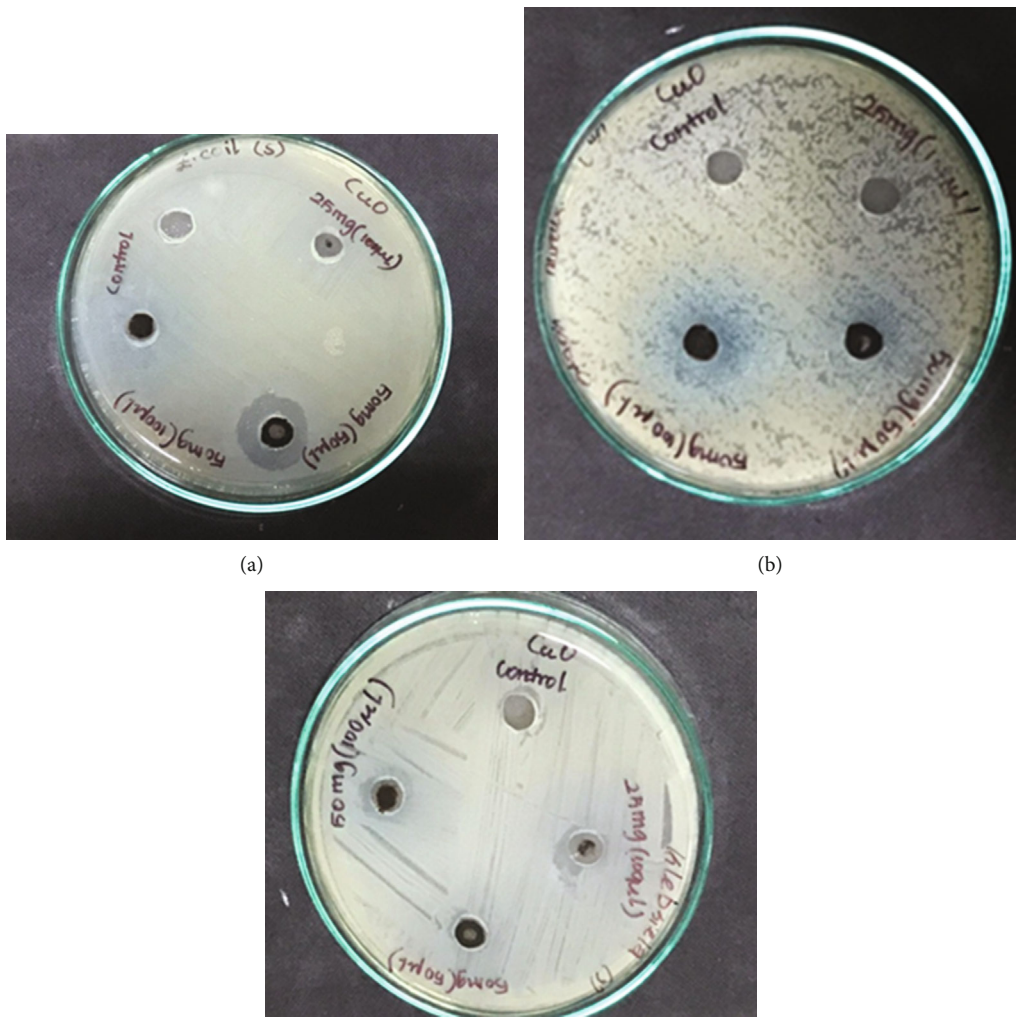
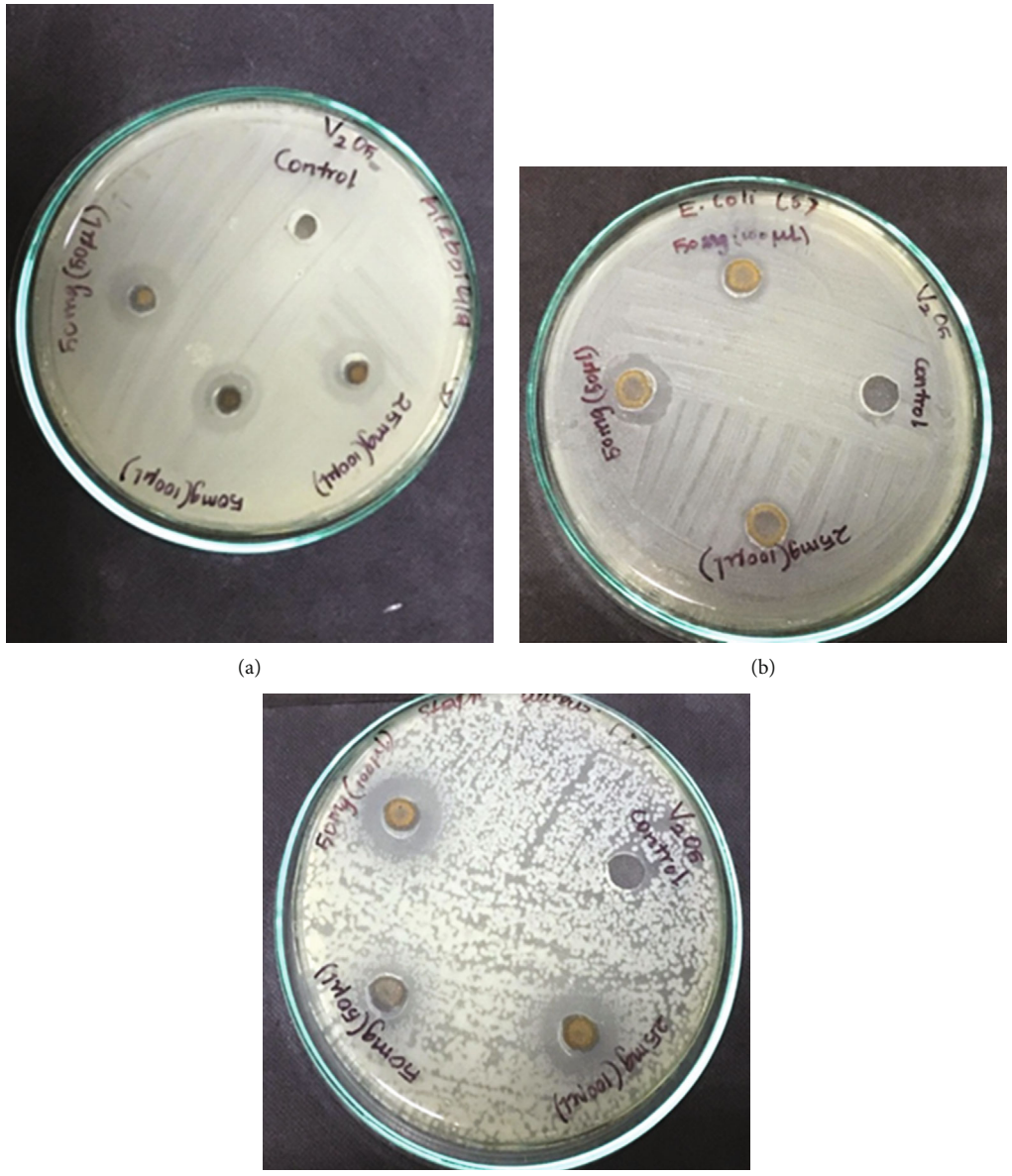


Figure 1: Bactericidal activity of CuO-NPs: (a) *E. coli*; (b) *S. aureus*; (c) *K. pneumoniae*.



(a) (b)

(c)

Figure 2: Bactericidal activity of V_2O_5-NPs: (a) *K. pneumoniae*; (b) *E. coli*; (c) *S. aureus*.

bacterial strains and exhibited the highest inhibitory zone against *E. coli* and *E. faecalis*. Similarly, Sivaraj *et al.* [34] also proved the bactericidal efficiency of CuO NPs synthesized using the leaf extract of *Tabernaemontana divaricate*, and it produced 17 mm sized inhibitory zone against *E. coli* at 25 μg/mL of concentration.

Vanadium pentoxide NPs inhibit the growth of all the strains which indicates the broad-spectrum toxic effect on the susceptible bacterial strains. It causes lethal effect observed with 11, 16, and 17 mm sized zone formation against *S. aureus*, and 12, 13, and 14 mm were found at the concentration 25 mg/50 μL, 25 mg/50 μL, and 50 mg/50 μL, respectively, against *K. pneumoniae*. For *E. coli*, inhibitory effect was observed as 8 mm at 25 mg/50 μL, 12 mm at 25 mg/50 μL, and 14 mm at 25 mg/50 μL. Kannan et al. [35] have demonstrated the antibacterial potency of V_2O_5 NPs against *P. aeruginosa*. The inhibitory effect occurred through the attachment of a negatively charged cell membrane with positively charged V^{5+} that assisted in the penetration of NPs into the bacterial cell which causes DNA damage and lysis of the bacteria. Aliyu et al. [36] stated that green synthesized vanadium oxide NPs prepared using *Moringa oleifera* leaf extract inhibit the growth of bacteria. Kannan *et al.* [35] also utilized *Andrographis paniculata* (leaf extract) as a chelating agent for the synthesis of V_2O_5 NPs through microwave-assisted method. These potential metal oxide NPs can be promising antibacterial agents to treat illness caused by drug-resistant foodborne pathogens.

3.2. Structural Characterization of V_2O_5 Nanoparticles. Among all the synthesized NPs, V_2O_5-NPs broadly showed bactericidal activity against all the selected pathogens. Thus,

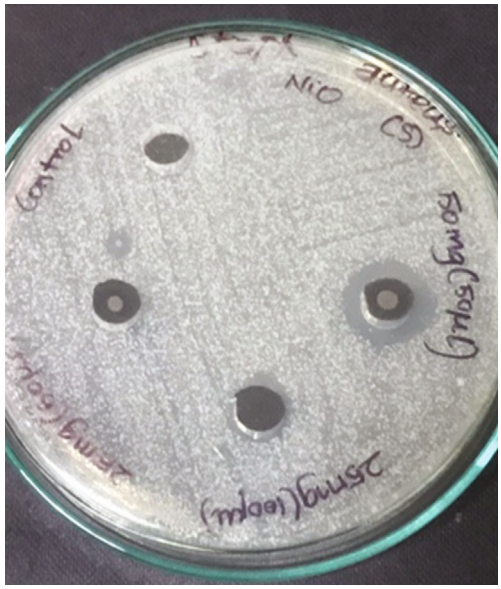


Figure 3: Bactericidal activity of NiO NPs against *S. aureus*.

further characterization studies were carried out for V_2O_5-NPs. The crystalline nature, functional groups, and morphological with elemental composition features were analyzed by XRD, FTIR, SEM with EDAX, and TEM analysis.

3.2.1. XRD Analysis of V_2O_5-NPs. XRD pattern of V_2O_5-NPs synthesized using extract of *Andrographis paniculata* as reducing and stabilizing agent is shown in Figure 4. The spectra showed sharp peaks in the 2θ angle region, indexed to (2 0 2), (0 1 1), (1 0 3), (2 0 2), (4 1 0), (2 0 5), (4 0 4), (4 0 6), (1 2 5), and (2 0 2) Miller index planes matched with JCPDS card no. 85-2422. The crystalline NPs exhibit orthorhombic structure, and the average crystallite structure was determined by Debye-Scherrer's equation, $D = K\lambda/\beta \cos\theta$, where "$D$" referred to mean crystallite size, "K" is shape constant, angular FWHM (Full Width at Half Maximum) is denoted as "β," and "θ" is the diffraction angle. The average crystallite size of V_2O_5 NPs was originated to be 20 nm. The lattice parameters a, b, and c were calculated by applying the specific formula for orthorhombic structure and the value for lattice constant $a = 9.946$, $b = 3.585$, and $c = 10.042$ Å. Similarly, Raj et al. [37] also stated the orthorhombic structure of V_2O_5 NPs synthesized by the chemical cum sonication method. The occurrence of sharp peaks in the XRD pattern indicated the excellent crystalline nature of the nanoparticle [38]. Alghool *et al.* [39] have recorded the orthorhombic structure of V_2O_5 NPs.

3.2.2. UV-Visible Spectral Analysis of V_2O_5-NPs. The absorbance spectrum of V_2O_5-NPs was observed between the wavelength 200 and 400 nm, and the result is shown in Figure 5. The colour changes from pale yellow to dark were owing to the phenomenon of Surface Plasmon Resonance (SPR), and the change in colour primarily confirmed the reduction of bulk materials to NPs by the plant extract [36]. Alghool et al. [39] reported the absorbance peaks at

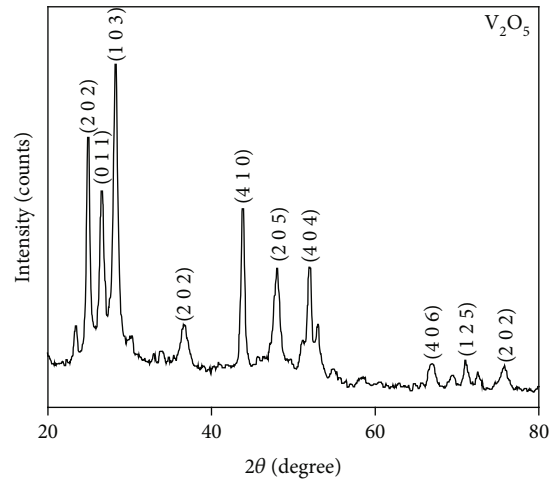


Figure 4: XRD pattern of V_2O_5 NPs

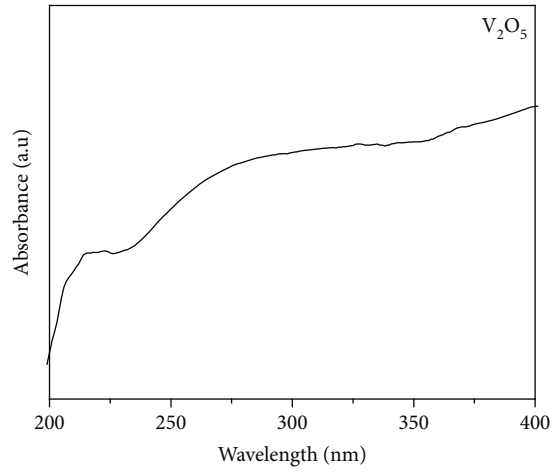


Figure 5: UV-Vis absorbance spectrum of V_2O_5 NPs.

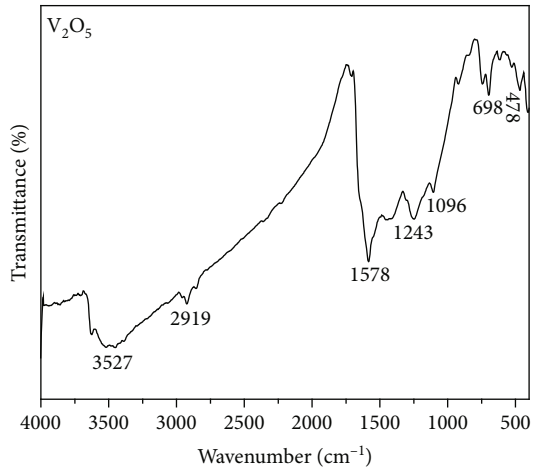


Figure 6: FTIR spectrum of V_2O_5 NPs.

234, 265, and 317 nm after band fitting by Gaussian functions using Origin 9.3 version software. Alghool *et al.* [39] have reported the existence of absorbance peak at 470 nm

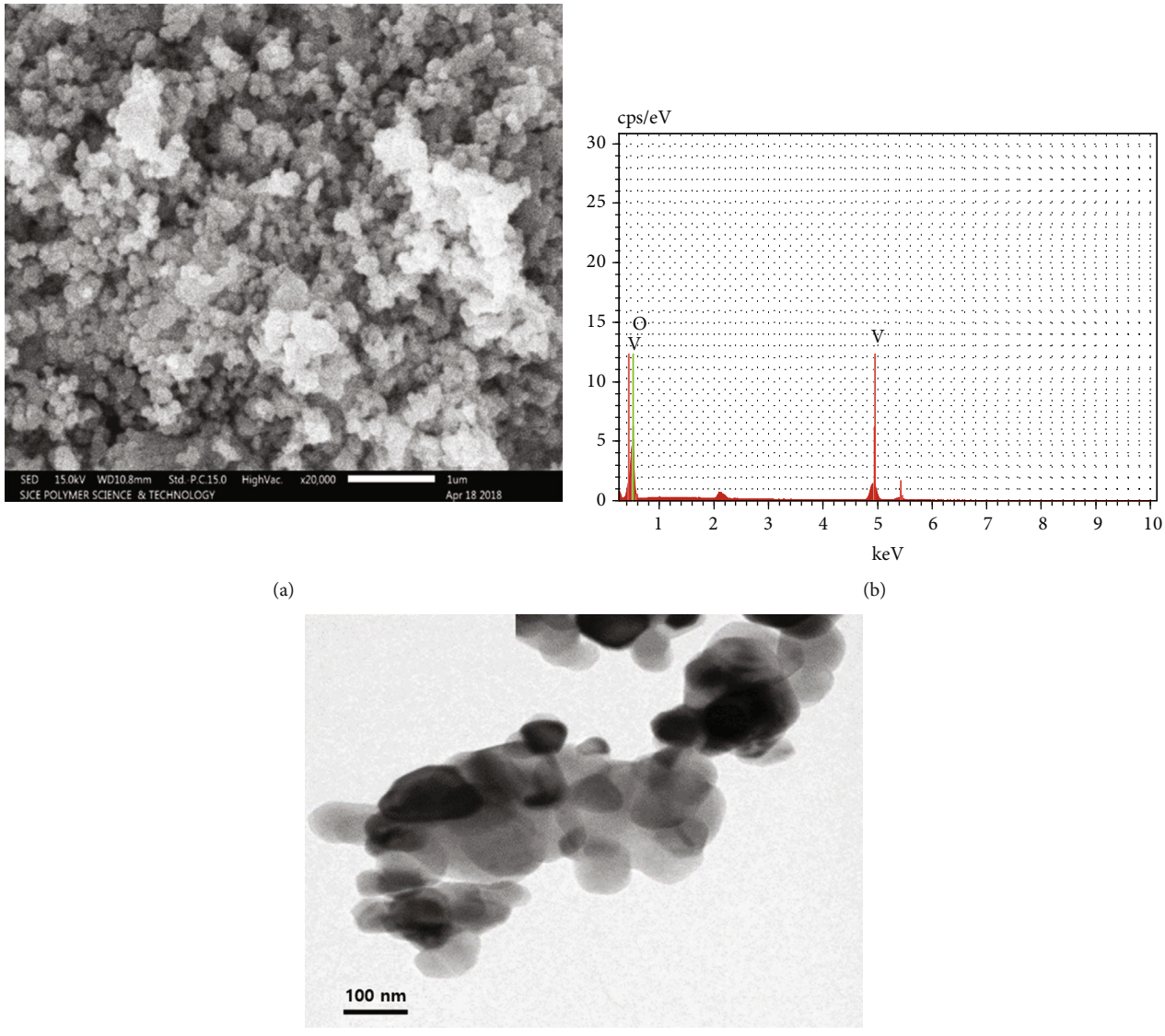


Figure 7: SEM (a) with EDX spectrum (b) and TEM (c) analysis of V_2O_5-NPs.

assigned to the transition ($n \longrightarrow \pi *$) of the V=O group to V_2O_5 NPs.

3.2.3. FTIR Spectrum of V_2O_5-NPs. The FTIR spectrum of V_2O_5-NP is pictured in Figure 6. The functional groups found in the V_2O_5-NP were identified by FTIR spectrum in the wavelength ranges from 4000 to 400 cm^{-1}. The absorption peaks 3527 cm^{-1} given to the hydroxyl group indicates presence of water molecules; 2919, 1578, and 1243 cm^{-1} band attributed to stretching of the CH_2 group, nitrogen containing group, and C-O stretched carboxylic acid which might be obtained from the plant extracts and acts as a reducing and capping agent of NP synthesis [36]. The band observed at 1096 cm^{-1} corresponds to other functional groups. The vanadium group presence was identified by the peaks observed in the ranges from 400 to 750 cm^{-1} [40]. This report assisted to confirm the presence of V_2O_5 NPs due to the presence of peaks at 698 and 478 cm^{-1}. The present result agreed with the outcome of Kannan et al. [35]. The authors synthesized V_2O_5 NPs via ultrasound-assisted method and reported that the functional group V-O-V vibrational stretching bond at 478 cm^{-1} indicated the formation of V_2O_5-NPs.

3.2.4. SEM and EDX Spectrum Analysis of V_2O_5-NPs. SEM analysis proved the morphological features of vanadium oxide NPs, and the micrograph is shown in Figure 7(a). The results exposed the cluster of spherical-shaped NPs. A similar finding was stated for V_2O_5 NPs developed by *M. oleifera* leaf extract by Aliyu et al. [36]. The morphology of the NPs mostly depends on the fabricating process. Farahmandjou and Abaeiyan [41] also studied the surface morphology of V_2O_5 NPs. Alghool *et al.* [39] have reported the agglomerated spherical shape of V_2O_5 NPs in their SEM analysis. The EDX spectrum of V_2O_5-NPs is given in Figure 7(b). The elemental analysis carried out by energy

dispersive X-ray spectroscopy examination confirmed the purity of green synthesized vanadium oxide NPs. The elemental composition of V_2O_5 NPs was found to be V (32.34%) and O (67.66%). The intense peaks for the vanadium compound were observed nearer to 0.15 keV and 4.9 keV.

3.2.5. TEM Analysis of V_2O_5-NPs. TEM micrograph of prepared V_2O_5 NPs is presented in Figure 7(c). The images showed the aggregated spherical particles of V_2O_5 [35]. The average particle size of V_2O_5 NPs was determined by ImageJ software, and the size was found to be 58 nm. The particle size might depend on the reaction period taken for bioreduction bulk vanadium to nanoscale V_2O_5 [39]. The TEM analysis revealed the patterns and distribution of crystalline particles [41].

4. Conclusion

Foodborne pathogens are responsible for a wide range of diseases that have serious consequences for human health and the economy. Metal oxide NPs (NiO, CuO, ZnO, MoO_3, and V_2O_5) were produced employing extracts from *Hydrangea paniculata*, *Plectranthus amboinicus*, and *Andrographis paniculata* as reducing agents in the current study. The crystalline and morphological characteristics of V_2O_5-NPs were demonstrated through characterization studies. Our study proved that the *E. coli*, *S. aureus*, and *K. pneumoniae* were more sensitive to V_2O_5-NPs. As a result, we concluded that the biosynthesized metal oxide NPs (NiO, CuO, ZnO, MoO3, and V_2O_5) were a promising antibacterial material against drug-resistant pathogenic bacteria, as a replacement for currently existing inefficient antibacterial drugs.

Data Availability

All data generated or analyzed during this study are included in the published article.

Conflicts of Interest

There is no conflict of interest.

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