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Sustainable Nanoparticle Synthesis Using *Tinospora cordifolia* (Giloy) Leaves Extracts: Evaluation of Antimicrobial Efficacy Against MultiDrug Resistant Bacteria and Optical Features

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Abstract: Sustainable synthesis, also known as environmentally benign synthesis, of nanoparticles, presents an energy and resource-efficient approach for the development of nanoparticles to advanced materials with considerable biomedical potential. This study focuses on the synthesis of zinc oxide (ZnO) nanoparticles using *Tinospora cordifolia* (Giloy) leaves, aqueous extract as a natural strengthening and stabilizing and natural reducing agent. The bioactive compounds present in the leaves extract facilitated the reduction of zinc ions (Zn^{2+}) to ZnO nanoparticles under optimized reaction conditions. For the characterization of nanoparticles, analytical tools were used like UV-Vis spectroscopy and X-ray diffraction (XRD) to determine the size of nanoparticles, their morphology, and crystalline structure. Green-synthesized ZnO nanoparticle's optical properties and their energy band gap were evaluated, which revealed an energy band gap of approximately 3.12 eV, which suggests their efficiency for photocatalytic and biomedical applications. The antimicrobial potential of the green-synthesized ZnO nanoparticles was assessed against a pathogen associated with waterborne infections which is Aeromonas hydrophila, showcasing multidrug-resistant (MDR) activity. The nanoparticles exhibited significant biocidal activity, with a zone of inhibition that is directly correlated to the size of nanoparticles and their concentration. The effectiveness of antimicrobial activity is attributed to the unique physicochemical properties of the ZnO nanoparticles, including a high surface area and size-dependent reactivity, which increases their interaction with bacterial cell membranes. This study brings to light the potential of *Tinospora cordifolia*-mediated green synthesis of ZnO which is a renewable and competent method for producing biofunctional ZnO nanoparticles. The encouraging antimicrobial activity, coupled with their tunable optical properties, positions these nanoparticles as valuable agents in combating multidrug-resistant bacteria, with further applications in nanomedicine, biosensing, and environmental remediation technologies.

Keywords: aqueous extract, bioactive compound, optical property, biomedical applications, antimicrobial activity

1. Introduction

The bioactive compounds found in herbal extract have been integral to traditional medicine (TM). The World Health Organization reported that approximately 80% of the global population relies on conventional or plant-based formulations like decoctions for therapies. Among these, the Menispermaceae family or moonseed family is a renowned name which consists of herbal plants like *Tinospora crispa, Cyclea peltata, Cocculus hirsutus, Cissampelos pareira*, and *Tinospora cordifolia*. Out of these, *Tinospora cordifolia* is a notable member, which is a widely used herb in Ayurvedic practices [1]. Historically as per ancient practices, this plant is famous by various names such as "Gulvel" in Marathi, "Amrutha", and "Guduchi" in Sanskrit, "Amrutha Balli" in Kannada and Hindi as "Gurcha". This herb has been mentioned in ancient Ayurvedic texts for its medicinal properties [2]. *Tinospora cordifolia* is a perennial plant, meaning it lives for several years, and it is also deciduous, shedding its leaves annually. It is characterized by long petioles, elongated, alternate, ex-stipulate, and pulvinate leaves that are slightly round with both the base and apex exhibiting partial twists. The stem is whitish-gray or creamy and covered with a thin bark [3]. In India,

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this herb thrives in a diverse range of soils, from acidic to alkaline, and even moderate levels of humidity work for its optimal growth [4]. As its Sanskrit name "Amrita" defines "immortality", this plant is traditionally used for enhancing youthfulness, longevity, and vitality. The plant parts like stems, leaves, and roots are used for the preparation of TM which helps in the treatment of ailments like dyspepsia, general debility, anemia, gonorrhea, fever, urinary disorders, viral hepatitis, and it acts as an immunity booster and provides anti-inflammatory, antibacterial, and antimicrobial property [1, 5]. It also protects from toxins thus helping in liver detoxification and protection. Some studies also claim it is an antioxidant in nature so it helps in the removal of free radicals and provides a neuroprotective effect in diseases like Alzheimer's. Over the period, research has also highlighted its potent immunomodulatory and antineoplastic properties [6]. Bioactive compounds which are derived from different parts of *Tinospora cordifolia* like leaves, stems, and roots have illustrated potential antioxidant activity in various studies conducted in vitro that signifies its pharmacological applications [7]. The phytochemicals found in herb extracts as well as herbal decoctions are widely utilized for their versatile therapeutic benefits, including anti-osteoporotic, anti-allergic, antispasmodic, cardioprotective properties, antipyretic, anticancer, and antidiabetic [8]. These pharmacological and pharmacodynamic activities underscore the herb's significant potential in therapeutic applications and modern medicine [9].

In nanotechnology, the process of sustainable synthesis or green synthesis marks an innovative strategy, emphasizing environmentally aware methodologies with the help of this energy consumption and toxic by-products can be minimized. This innovative and sustainable approach is particularly pivotal in confronting and overcoming the exacerbating threat of multidrug-resistant bacterial species, which poses an intimidating challenge in contemporary healthcare [10]. Capitalizing natural resources, such as plant devotions and extracts, for nanoparticle synthesis not only aligns with the ideology of environmental guardianship but also opens the door to the development of alternative care regimens [11]. Sustainable synthesis of metal and metal oxide nanoparticles like AuNPs, AgNPs, CuO NPs, ZnO NPs, TiO2, SiO2 has extensively used in which different plant extracts like *Azadirachta indica*, *Moringa oleifera*, *Aloe vera*, *Ocimum sanctum*, *Camellia sinensis*, *Phyllanthus emblica*, *Eclipta alba*, *Musa acuminata,* and *Oryza sativa'*s husk extract as a stabilizing and reducing agent due to the richness of phytochemical composition and these are eco-friendly. Among these plants, *T. cordifolia* revealed itself as a promising candidate for the synthesis of nanoparticles due to the higher content of phytochemical constituents like terpenoids, flavonoids, and alkaloids. These phytochemicals hold a broad spectrum of pharmacological properties and showcased remarkable results in in vitro studies. Tinosporin, a triterpenoid present in *T. cordifolia, showed* remarkable results by inhibiting the growth of cancerous cells in vitro studies. Additionally, it also protects against toxins and damage caused by alcohol. A similar type of antioxidant activity is represented by the phenolic compounds that are present in Giloy. Steroids showed both anti-inflammatory and antidiabetic activity. Sitosterol, a steroid compound, reduces inflammation and also regulates blood sugar levels. Berberine, an alkaloid, showed antimicrobial as well as antioxidant and anti-inflammatory activity [12]. Gallic acid, another phenolic compound found in *T. cordifolia* plant extract, showed antibacterial, antiviral, anticancer hepatoprotective, antidiabetic, and neuroprotective effects [12, 13]. By leveraging the pharmacological properties of *T. cordifolia* in eco-conscious synthesis, this approach bridges traditional and modern medicinal paradigms, embodying a vital step forward in the pursuit of sustainable and impactful healthcare innovations against normal and multidrug-resistant bacterial infections [14].

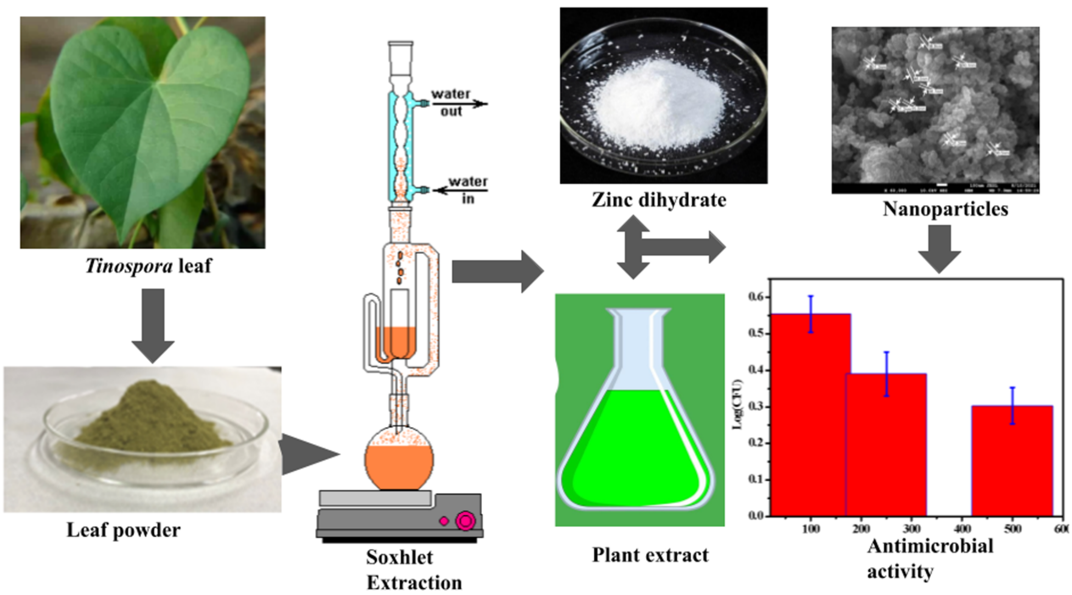
Multiple drug resistance is a condition in which microorganisms like bacteria, fungi, and other parasites develop resistance against drugs that are used against them for the treatment of infection. This develops through natural selection, gene mutation, horizontal gene transfer, and vertical gene transfer. Additionally, some other mechanisms like biofilm formation, influx and efflux pumps, and the use and misuse of drugs also contribute to the development of resistance genes. The escalating prevalence of resistant genes in bacterial species towards standard antibiotics due to their use and misuse necessitates an immediate paradigm shift toward the refinement of alternative therapeutic techniques. Concerning this context, sustainable synthesis emerges as a groundbreaking frontier, capitalizing on biological resources to generate eco-conscious and impactful nanomaterials [14]. The deployment of *Tinospora cordifolia* in nanoparticle synthesis is particularly intriguing, given its rich repository of biologically active phytochemicals exhibiting significant antioxidant and antimicrobial properties. Due to the richness of phytochemical constituents, *Tinospora cordifolia* provides a multi-functional strategy for combating multidrug-resistant bacteria and the infections caused by these [15]. By incorporating the phytochemicals in metal oxide nanoparticles, this activity can be enhanced up to several folds. Sustainable synthesis of nanoparticles and incorporation of plant extract provides a large array of applications like dye removal, environmental remediation, wastewater removal, heavy metals removal, and antiviral and antibacterial activity. Different metal oxide nanoparticles like silver, gold, copper, and zinc when used with the different phytochemical compounds helped in achieving the biocompatibility. The aqueous fruit extract of nutmeg when used for the green synthesis of ZnO nanoparticles represented a large array of applications in environmental and biological applications. Another study showcased the effective antimicrobial activity of green-synthesized ZnO nanoparticles using *Azadirachta indica* (Neem) leaf extract against these bacterial species *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa.* Green-synthesized MgO and CuO nanoparticles showed effective results against the adsorption of heavy metals like cadmium and chromium and lead removal and environmental remediation. Biologically influenced nanoparticle synthesis effectively responds to the antibiotic resistance predicament while amalgamating eco-friendly and sustainable solutions with advanced therapeutic breakthroughs [16].

Zinc oxide (ZnO) nanoparticles demonstrate significant antibacterial activity against both resistant and non-resistant strains of bacteria. When these nanoparticles penetrate the bacterial cell membrane, this binding leads to cellular content leakage. When nanoparticles bind to cellular membranes, they show electrostatic interactions and the sharp edges of nanoparticles easily disrupt the bacterial membrane structure which leads to an increase in the cellular membrane permeability and causes apoptosis. However, the resistant bacterial species have modified the outer membrane, but ZnO nanoparticles easily bypass this modified membranal barrier through direct penetration in the membrane. When ZnO nanoparticles enter inside the bacterial cytoplasm, they release Zn^{2+} ions, and these ions bind with different enzymes, proteins, and nucleoid regions leading to disruption of replication and metabolic pathways. Its experimental evidence was showcased by TEM, FE-SEM, and Live/Dead assay studies [17].

The intimidating escalation of resistant genes in bacteria due to the use and misuse of antibiotics illustrates a precarious worldwide health challenge, compelling the experimentation of revolutionary restorative alternatives [17, 18]. Sustainable synthesis, an emerging paradigm in nanotechnology, offers an eco-friendly and carbon-neutral solution by incorporating biological resources to fabricate nanoparticles with immanent antimicrobial and biocidal properties [19, 20]. Harnessing plants like *Tinospora cordifolia*, known for their medicinal and multifaceted phytochemical compounds and resilient pharmacokinetic and pharmacodynamic characteristics, provide a coupled advantage in tackling multidrug-resistant bacteria while complying with eco-compatible and organic practices [21, 22]. This method not only meets the urgent need for alternative treatments but also highlights the collaboration between traditional medicinal knowledge and modern scientific progress in tackling one of healthcare's greatest challenges [23]. Nanoparticles offer key properties like optoelectronic features such as fluorescence, UV-Vis absorption, and energy bandgap and illustrate the critical interpretation of their functional and structural characteristics, which are significant for therapeutic applications [24]. In the case of fabricated nanoparticles like plant-derived nanoparticles, for example, *Tinospora cordifolia* (Giloy) that is used in this study, the optoelectronic features illustrate a significant interpretation for their electronic, structural, and photonic behavior [21, 25]. For detailed investigation of nanoparticles like their size, surface interactions, and morphology, UV-Vis absorption spectra are significant while the photochemical compounds and their antimicrobial efficiency can be easily estimated with the help of fluorescence characterization [26, 27]. Moreover, the energy band gap determines the nanoparticle's light and reactivity interaction, which are significant for their effectiveness in therapeutic settings [5, 28, 29]. A detailed understanding of these optical properties is indispensable for refining the design of nanoparticles and maximizing their health-promoting potential, particularly in confronting the escalating challenge of drug resistance [30].

The main objective of the current study is to use the richness of bioactive components present in *Tinospora cordifolia* or Giloy leaves to develop sustainably synthesized nanoparticles as carbon-neutral and sustainable solutions for tackling multidrug-resistant genes and bacteria. Compared to the conventional synthesis process, which relies on chemical reducing agents, the use of hazardous solvents in such methods poses a significant threat to both human health and the environment [31, 32]. The physicochemical properties like temperature and pressure require prolonged reaction time and exposure. The base of chemical synthesis is often a non-biodegradable by-product which results in harmful residue formation, and it further requires additional steps for the disposal or the treatment which is quite a complex and expensive process [33, 34]. However, biological sources are eco-friendly and energy efficient as these often require room temperature and normal atmospheric pressure. Advancements in the sustainable synthesis process of nanoparticles provide controlled stability, and morphology reduces or minimizes toxic by-products and provides enhanced functional properties. By deploying this medicinal plant, the investigation strives to generate eco-conscious nanomaterials that offer an eco-friendly and groundbreaking application to deal with the pressing challenge of multidrug-resistant bacteria [29, 30, 35]. For the evaluation of antibacterial efficiency, the green-synthesized nanoparticles were tested against the MDR species that were isolated from wastewater samples. The significant results that will be obtained from these nanoparticles will provide insights into their antimicrobial potential, and the estimation of their optical properties will shed light on their structural and functional characteristics. Figure 1 provides an overview of research work conducted. The current research not only signifies the principles of sustainable practices but also contributes to the development of advanced, biocompatible solutions to combat conventional antibiotic resistance problems. Unlike the other studies that use simple techniques for extraction of phytoactive compounds, this research employs Soxhlet-based extraction techniques for aqueous as well as chloroform extracts, to ensure maximum controlled and efficient process. Furthermore, the additional step involved maintaining the extract to remove debris this was used to synthesize sustainable ZnO nanoparticles, and then these nanoparticles were tested against waterborne multidrug-resistant bacteria and to broaden the other areas like optical activity.

Figure 1
Giving an overview of the conducted research work



2. Materials and Methods

2.1. Leaves collection and processing

Fresh leaves of *Tinospora cordifolia* were collected from the Medicinal and Aromatic Plants Section, CCS Haryana Agricultural University, Hisar, India. To maintain the quality of raw material, leaves without any physical defect or disease and pathogen-free were deliberately chosen. Then, these leaves were brought up in the laboratory where they were thoroughly rinsed/cleaned under running tap water for the removal of surface impurities like dirt, dust, fertilizer residues, insect eggs, larvae, etc., further followed by washing with double distilled water to eliminate any residual particulate matter. The thoroughly rinsed leaves were then air-dried in a shaded environment using an ambient temperature of 25 ± 2 °C for 6–7 days to preserve the phytochemical compounds present in the leaves. After completion of the drying process, these leaves were put in the laboratory-grade grinder for proper grounding into a fine powder. To ensure a consistent particle size, this leaves powder further passed through Sethi Standard Test Sieves with a particle size of BSS 156, 0.106 mm for uniform particle size distribution using the sieve method. This step ensures the proper size of the ground powder for analysis. For further experimentation, this leaf powder was stored in an airtight container at room temperature [1].

2.2. Bioactive compounds extraction using Soxhlet apparatus

With the help of the Soxhlet apparatus purchased from Jain Scientific Glass Works (18782), this fine leaf powder was subjected to aqueous extraction. For this extraction, 5 grams of powdered leaves were weighed and placed in the Soxhlet extraction unit with 100 mL of triple distilled water and chloroform (with DMSO) as the solvent. The process was carried out for 8 h, ensuring optimal extraction of phytoactive compounds for pharmacological purposes for compounds such as alkaloids, flavonoids, and polyphenols. The extract was then collected and filtered further to remove residual debris. Further filtration was carried out using centrifugation with the help of MPW-350R at 5000 rpm for 15 min. After the filtration, the extract was kept in a sterile container at 4°C for further analysis. Figure 2 illustrates the sequential process of *Tinospora cordifolia* leaf collection and further Soxhlet extraction process [1].

2.3. Sustainable synthesis of nanoparticles

The phytochemicals, such as polyphenols, alkaloids, and flavonoids, present in the leaf extract of *Tinospora cordifolia* (Giloy) serve as stabilizing and reducing agents in the sustainable synthesis of nanoparticles. The plant extract, when used for this synthesis, yields 0.010204 g at 2% (w/w), 0.020833 g at 4%, and 0.031914 g at 6%, respectively. During the synthesis process, these phytoactive compounds donate electrons to zinc ions (Zn^{2+}) derived from zinc salts, facilitating their reduction to elemental zinc. The process involves multiple steps, starting with the reduction of Zn^{2+} ions to ZnO, followed by nucleation and controlled growth of ZnO nanoparticles, which are further stabilized by the bioactive compounds acting as capping agents. Utilizing zinc salts, like zinc acetate or zinc nitrate, is essential since the size, shape, and quality of the nanoparticles are greatly influenced by the precursor selection. The ideal conditions for green synthesis of ZnO nanoparticles, including pH, reaction time, temperature, and concentrations of both the zinc precursor and the leaf extract, are critical for achieving desirable particle characteristics. For example, an alkaline range, specifically pH ~10, enhances the hydrolysis of the zinc salt and helps in the stabilization of nanoparticle formation and the reduction process even without the formation of secondary reactants. Likewise, if the concentration of leaf extract like 2–8% v/v and the precursor of zinc is balanced carefully it also helps in ensuring uniform particle size of ZnO and prevents the aggregation of nanoparticles. The reaction times of around 2 h allow for complete reduction and stabilization without secondary reactions. Similarly, the concentration of the extract and zinc precursor for example 0.01–0.1 M must be carefully balanced to ensure uniform particle size and prevent aggregation. Analytical techniques, such as UV-Vis spectroscopy, scanning electron microscopy (SEM), and X-ray diffraction (XRD), are employed to get insights into ZnO nanoparticles to validate their structure, size, and optical properties. These nanoparticles demonstrate significant antimicrobial and antioxidant properties, highlighting their potential for various biomedical and environmental applications. By minimizing harmful by-products and conserving energy, this environmentally friendly synthesis process using *Tinospora cordifolia* is scalable, beneficial to the environment, and aligned with the principles of green chemistry. The process utilizes the rich phytochemical profile of T. cordifolia to combat the rising challenge of multidrug-resistant bacteria and to develop sustainable nanomaterials for wider therapeutic applications. By optimizing and validating the synthesis

Figure 2
Showing the leaves collection and further processing using Soxhlet

process, this research advances reproducible and environmentally sustainable methods for producing nanoparticles, connecting traditional medicinal knowledge with contemporary nanotechnology. The synthesis of ZnO nanoparticles requires optimization of key parameters for efficiency and effectiveness. For synthesis, a temperature of approximately 80°C is ideal. This temperature prevents the degradation of biologically active compounds in the extract while providing sufficient energy for the reduction of Zn^{2+} ions and improving the crystalline structure of ZnO nanoparticles. The optimal alkaline conditions for synthesizing ZnO nanoparticles occur at a pH of 10. This pH level stabilizes the formation of ZnO and enhances the hydrolysis of zinc precursor salts. To ensure effective nanoparticle formation, the pH can be adjusted using bases such as NaOH. The ideal concentration of leaf extract is between 2% and 10% (either by volume or weight). Higher concentrations offer more reducing and stabilizing agents, but using too much can lead to the formation of larger or aggregated nanoparticles. Therefore, it is essential to find a balance to achieve uniform nanoparticle size. The zinc precursor commonly used is zinc dihydrate (H_2OZn^{2+}), which has an optimal concentration range of 0.01 M to 0.1 M. This ensures there are enough Zn^{2+} ions for nanoparticle synthesis. This ensures there are enough Zn^{2+} ions for nanoparticle synthesis. However, very high concentrations may lead to larger particle sizes or aggregation. This duration allows for the complete reduction and stabilization of nanoparticles. To prevent unwanted secondary responses, prolonged reaction times should be monitored carefully. High-quality ZnO nanoparticles with desired characteristics can be produced by adjusting the synthesis process parameters. Giloy leaf extract's biologically active components have stabilizing properties in addition to their decreasing activity. By creating a protective coating around the freshly produced ZnO nanoparticles, these substances stop them from forming or aggregating. This stabilization ensures that the nanoparticles remain uniformly dispersed, preserving their desired size and morphology. The stabilizing compounds derived from plants aid in preserving the ZnO nanoparticles' colloidal stability and structural integrity in solution. The stabilized ZnO nanoparticles synthesized with Giloy extract have numerous potential applications, including antimicrobial treatments, drug delivery, biosensing, and environmental remediation. Their distinct physicochemical properties, including size-dependent reactivity and a high surface area, make them particularly effective against microbial resistance and enhance the effectiveness of various biomedical and industrial processes.

2.4. Characterization of synthesized nanoparticles

After the sustainable synthesis, the nanoparticles are characterized using different analytical techniques to assess their shape, structure, and other morphological properties. Different analytical techniques like UV-Vis spectroscopy, surface plasmon resonance (SPR), Fourier-Transform Infrared Spectroscopy (FTIR), XRD, and SEM were used for characterization of nanoparticles. For the identification of absorption peaks of synthesized nanoparticles, a pivotal technique like UV-Vis spectroscopy was used for confirming ZnO nanoparticle formation. With the help of this technique, the other properties like optical properties investigation along with absorption spectra help in confirmation of nanoparticle sizes. Crystallinity of nanoparticle structures was identified with the help of XRD, and SEM is used to visualize the particle size and morphology.

2.5. Surface plasmon resonance (SPR)

ZnO nanoparticles display a characteristic absorption peak in the UV region, generally around 370 nm, which varies based on their size and morphology. The intensity and position of this peak provide insights into particle stability and uniformity.

2.6. Energy band gap

The Tauc plot derived from the UV-Vis data also helps identify the optical band gap of ZnO nanoparticles, which is important for comprehending their electronic and photocatalytic properties. A characteristic band gap for ZnO nanoparticles ranges between 3.12 eV, demonstrating high optical transparency and conceivable applications in optoelectronic and photonic devices.

2.7. FTIR (Fourier-Transform Infrared Spectroscopy)

FTIR spectroscopy is a crucial analytical tool for identifying the functional groups in the *Tinospora cordifolia* (Giloy) extract involved in the synthesis and stabilization of ZnO nanoparticles. This technique provides insight into the chemical interactions between the bioactive compounds in the extract and the nanoparticle surface. FTIR spectra reveal specific absorption bands corresponding to functional groups such as hydroxyl (-OH), carbonyl (C=O), amine (-NH), and phenolic groups, which are responsible for reducing Zn^{2+} ions and stabilizing the ZnO nanoparticles.

2.8. X-ray diffraction (XRD)

XRD analysis serves as a critical tool for elucidating the crystallinity, phase composition, and structural integrity of ZnO nanoparticles synthesized via *Tinospora cordifolia* (Giloy) extract. The distinct diffraction peaks in the XRD spectrum signify the crystalline nature of the nanoparticles, with sharp and intense peaks corresponding to well-defined structures and broader peaks indicating smaller particle sizes or partial amorphousness. By matching the XRD pattern with standard reference data (e.g., JCPDS card no. 36-1451), the hexagonal wurtzite structure of ZnO is confirmed. Additionally, the average crystallite size is estimated using the Debye-Scherrer equation based on the full width at half maximum of the prominent diffraction peak. XRD further verifies the absence of secondary phases or impurities, highlighting the high purity of the synthesized nanoparticles. This comprehensive structural characterization ensures that the ZnO nanoparticles possess the requisite crystallinity and phase uniformity for applications in diverse biomedical, environmental, and industrial fields.

2.9. Scanning Electron Microscopy (SEM)

SEM serves as a pivotal technique for elucidating the size, surface morphology, and structural intricacies of ZnO nanoparticles synthesized via *Tinospora cordifolia* (Giloy) extract. The high-resolution, three-dimensional imaging capabilities of SEM enable precise visualization of nanoparticle shape, including spherical, rod-like, or hexagonal geometries, as well as surface texture and homogeneity. This method is invaluable for assessing particle size distribution and identifying potential agglomeration, both of which are critical parameters influencing the nanoparticles' functional performance in various applications. Uniformly dispersed nanoparticles with consistent morphology often exhibit enhanced antimicrobial efficacy and optical properties.

2.10. Antibacterial activity testing

Selection of MDR bacterial strains: The study utilized *Aeromonas hydrophila subsp. hydrophila* (Accession no.

CP000462), an MDR bacterial strain isolated from a wastewater treatment plant in Hisar, Haryana, India [16–18]. The bacterial culture was maintained in Mueller Hinton Broth at 37 °C with 180 rpm agitation for 24 h. Antibacterial susceptibility was evaluated using green-synthesized *Tinospora cordifolia*-mediated ZnO nanoparticles through in vitro methods, including the disc diffusion assay and Minimum Inhibitory Concentration (MIC) testing. Bacterial cultures standardized to 10^6 CFU/mL were uniformly spread on sterilized Mueller Hinton Agar plates using a sterile spreader. Plates were pre-incubated at 37 °C to ensure the absence of contamination. Wells with a 5 mm diameter were created using a cork borer, and after allowing the bacterial suspension to dry for 20 min, each well was loaded with 10 µL of green-synthesized ZnO nanoparticles at varying concentrations (2%, 4%, 6%, and 8% w/v). Plates were then incubated at 37 °C to evaluate the antibacterial activity. All experiments were performed in triplicate for consistency. Nutrient media were procured from Himedia. The antibacterial efficacy of the nanoparticles was determined by measuring the zone of inhibition at regular intervals, and the results were plotted graphically to assess the effectiveness of the green-synthesized ZnO nanoparticles against MDR strains.

2.11. Statistical analysis

The results obtained were expressed as means ± standard errors, with statistical analysis performed using SPSS version 22. The graphical representation of the results was generated using Origin Pro 8.5, providing a clear and concise visualization of the antibacterial activity at various concentrations. This approach ensures that the data analysis is both statistically sound and scientifically rigorous.

3. Results

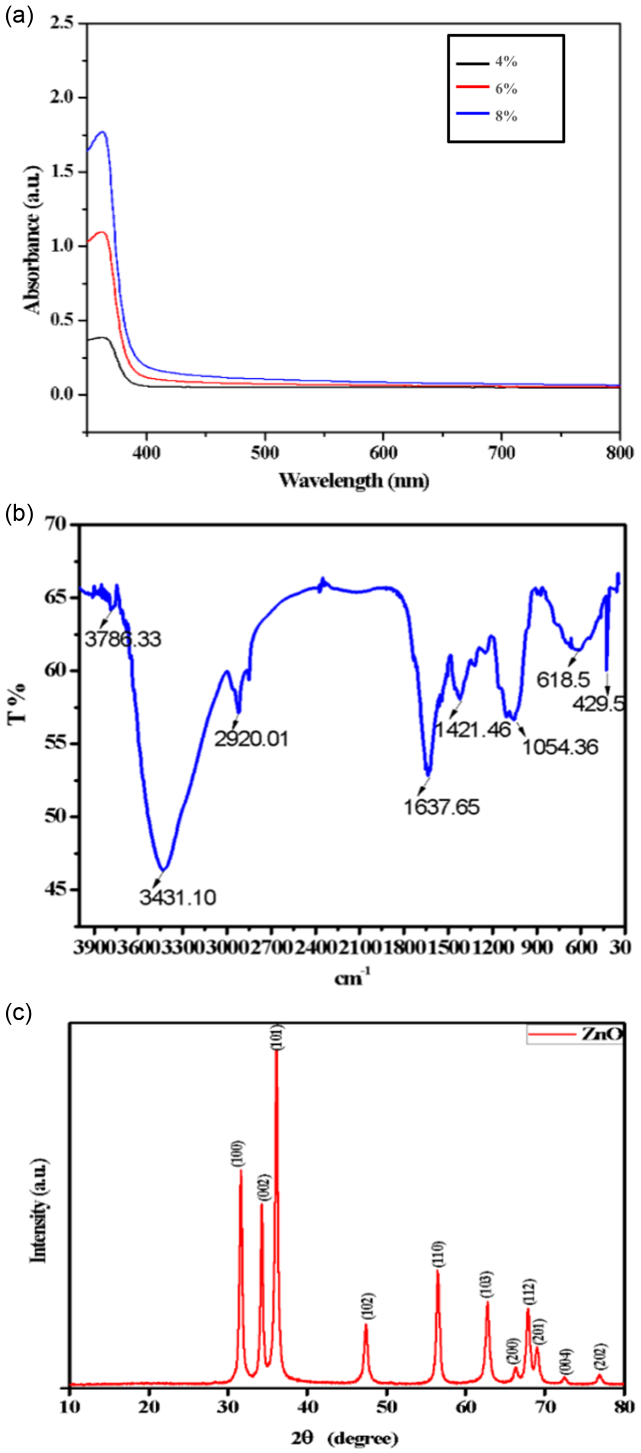
The synthesis and characterization of *Tinospora cordifolia*-mediated ZnO nanoparticles were meticulously analyzed through UV-Vis spectroscopy, capturing their optical properties across a wavelength range of 200–800 nm. A prominent absorption peak, typically around 360 nm, was observed, indicating the SPR phenomenon and confirming the successful formation of ZnO nanoparticles, as shown in Figure 3a–e, which presents results from various analytical techniques.

Notable shifts in the peak position or variations in intensity were carefully recorded, offering valuable insights into the stability, uniformity, and potential size distribution of the nanoparticles. These spectral characteristics also highlight interactions between the nanoparticles and bioactive compounds present in the plant extract. Such comprehensive analysis underpins the efficacy, reproducibility, and scalability of the green synthesis process.

The spectroscopic properties of green-synthesized ZnO nanoparticles utilizing *Tinospora cordifolia* extract at different v/v % concentrations (4%, 6%, and 8%) are demonstrated by the UV-Vis absorbance spectrum. The absorbance peaks, which are clearly visible below 400 nm, are consistent with ZnO nanoparticles' distinctive SPR, demonstrating that these particles are nanoscale in nature. The intensity of the peaks gets higher as the v/v% of extract increases, which indicates that the presence of more bioactive phytochemicals, which function as stabilizing and reducing agents, has led to improved nanoparticle formation and smaller particle sizes. The peak shows less strength at 4% v/v, which could indicate a greater particle size or a comparatively lesser yield. On the other hand, the intensity increases sharply and uniformly at 6% and 8% v/v, indicating increasing nanoparticle concentration. The peaks' broadness demonstrates a slight size polydispersity, while the spectrum's flattening beyond 400 nm emphasizes ZnO's effectiveness in blocking UV light and its low visible scattering. These results demonstrate that the optical

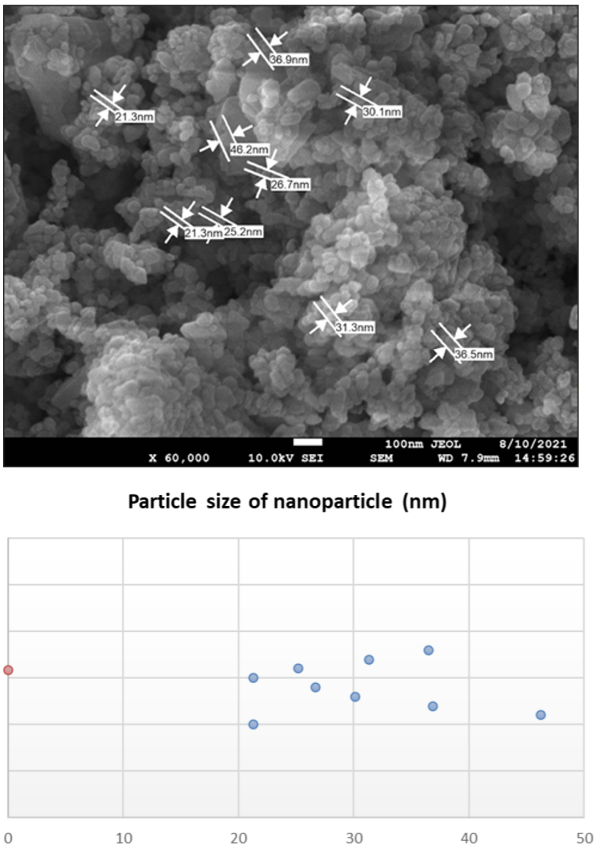
Figure 3

(a) The UV-Vis absorbance spectrum of synthesized nanoparticles of different concentrations, (b) FTIR analysis and (c) XRD analysis of *Tinospora cordifolia* leaf extract's nanoparticle, (d) SEM analysis of green-synthesized nanoparticles using *Tinospora cordifolia* leaf extract, and (e) the particle size of synthesized nanoparticles



(d)

(e)



characteristics, yield, and size distribution of ZnO nanoparticles are significantly influenced by the extract's concentration, which makes them appropriate for uses including UV protection and antibacterial activities.

Peaks associated with ZnO stretching vibrations, typically observed around 400–500 cm^{-1}, confirm the successful synthesis of ZnO nanoparticles. The FTIR spectrum confirms the presence of functional groups such as hydroxyl, carbonyl, and aromatic groups from the *Tinospora cordifolia* extract, which act as reducing and stabilizing agents during the green synthesis process. Additionally, the metal-oxygen vibrations validate the formation of ZnO nanoparticles. To confirm the presence of specific photoactive compounds in the extract, we used UHPLC-MS/MS (Agilent, Central Instrumentation Library), where the compound most closely related to gallic acid was detected. This bioactive compound showed effective results against MDR bacteria.

The diffraction peaks at 2θ values of approximately 31.7°, 34.4°, 36.2°, 47.5°, 56.6°, 62.8°, 66.4°, 68.0°, and 72.6° correspond to the planes (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202), respectively. The absence of additional peaks indicates the purity of the synthesized ZnO nanoparticles, with no significant contamination or secondary phases present. These values match the hexagonal wurtzite structure of ZnO. This XRD analysis confirms the successful synthesis of highly crystalline, phase-pure ZnO nanoparticles with a hexagonal wurtzite structure. The observed crystallinity and purity highlight the efficiency of the green synthesis approach, utilizing *Tinospora cordifolia* leaf extract.

The SEM analysis verifies the successful green synthesis of ZnO nanoparticles using *Tinospora cordifolia*. The observed morphology and particle size (ranging from 21.3 nm to 46.2 nm) suggest a promising nanomaterial with potential applications in antimicrobial, antioxidant, and other biomedical fields. The relatively uniform particle size distribution and morphology highlight the efficiency of the plant-based synthesis method.

When the synthesized nanoparticles were used for the antimicrobial activity testing, it provided significant outcomes. Here, *Aeromonas hydrophila subsp. hydrophila* is a Gram-negative, rod-shaped, facultatively anaerobic bacterium that is commonly found in aquatic environments. It is an opportunistic pathogen known to infect both humans and animals and is often linked to waterborne diseases and outbreaks. In humans, this bacterium can cause gastroenteritis, wound infections, and, in severe cases, septicemia [16].

This subspecies is a significant concern in aquaculture, as it can lead to conditions like hemorrhagic septicemia in fish, resulting in substantial economic losses. Its high adaptability to various environmental conditions and ability to form biofilms make it resilient and difficult to eliminate. Furthermore, *A. hydrophila subsp. hydrophila* is increasingly resistant to traditional antibiotics, highlighting the urgent need for alternative treatment strategies, such as the use of plant-based nanoparticles, to address infections caused by this pathogen.

The research results clearly demonstrate that green-synthesized nanoparticles derived from *Tinospora cordifolia* extract and ZnO nanoparticles exhibit distinct antibacterial properties, as shown in Figures 4 and 5, respectively. Significant antibacterial activity was demonstrated by the ZnO nanoparticles, which reduced CFU in a concentration and time-dependent manner. The log CFU showed relatively small efficacy at 100 ppm, decreasing from 0.810 at 10 h to 0.424 at 18 h. ZnO nanoparticles demonstrated increased activity at higher doses, such as 250 ppm and 500 ppm, with log CFU values of 0.405 and 0.390 at 18 h, respectively. These findings demonstrate the efficiency of ZnO nanoparticles, but they also imply that greater concentrations are required to produce significant bacterial suppression. In contrast, at every measured dose, the green manufactured nanoparticles made from *Tinospora cordifolia* extract performed better than ZnO nanoparticles. Greater efficacy was seen at 100 ppm, as the log CFU decreased from 0.554 at 10 h to 0.297 at 18 h. The decrease in activity appeared more prominent at 250 ppm, where log CFU decreased from 0.390 at 10 h to 0.105 at 18 h. *Tinospora* nanoparticles at 500 ppm exhibited almost total suppression, with a log CFU of 0.099 after 18 h. The bioactive substances in the plant extract, which probably strengthen the nanoparticles' antibacterial property, may be responsible for the green nanoparticles' increased effectiveness.

Significant increases in antibacterial activity for both types of nanoparticles have been demonstrated by statistical analysis as concentrations increase. On the other hand, *Tinospora* nanoparticles continuously displayed lower standard errors, suggesting that their effects were more precise and repeatable. For both nanoparticles, the decrease in CFU with time (from the time of inoculation till 24 h) was statistically significant; however, *Tinospora* showed a more noticeable time-dependent trend. This implies that both nanoparticles have bacteriostatic effects at first, followed by longer-lasting bactericidal behavior. In addition, the information demonstrates that *Tinospora* nanoparticles have a lower MIC and MBC value than ZnO nanoparticles, which is indicative of their greater potency. Similar findings were reported by Kahsay et al. [36], highlighting the enhanced antibacterial potential of green-synthesized ZnO nanoparticles due to their bioactive capping agents.

The correlation between the optical properties of green-synthesized ZnO nanoparticles and their antibacterial efficacy is intricately linked to their size, morphology, and surface functionalities, all of which are reflected in their optical behavior. The pronounced absorbance observed in the UV-Vis spectrum,

Figure 4
Antimicrobial activity of nanoparticles at different time intervals and concentrations

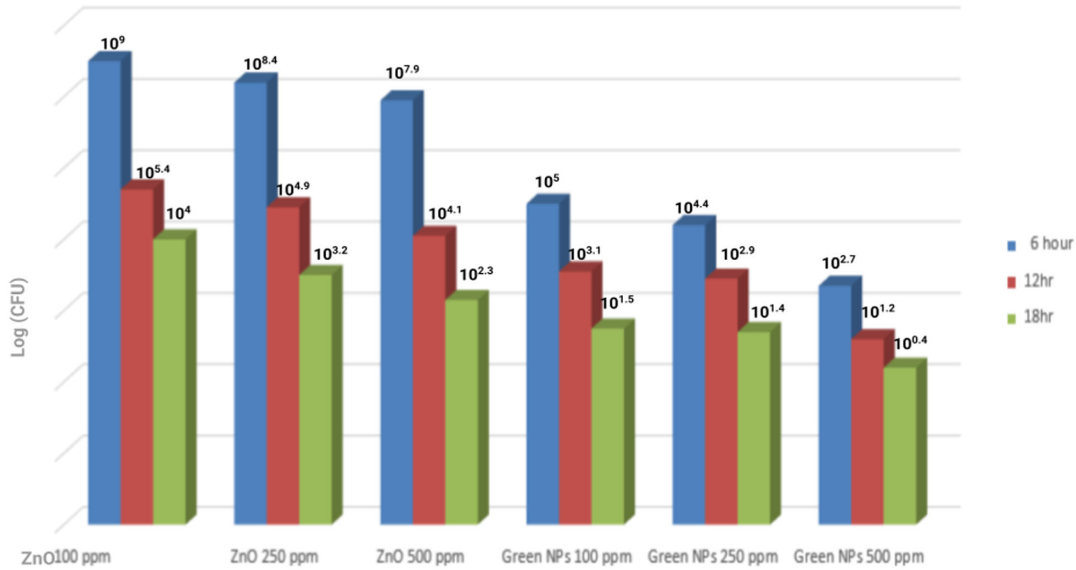
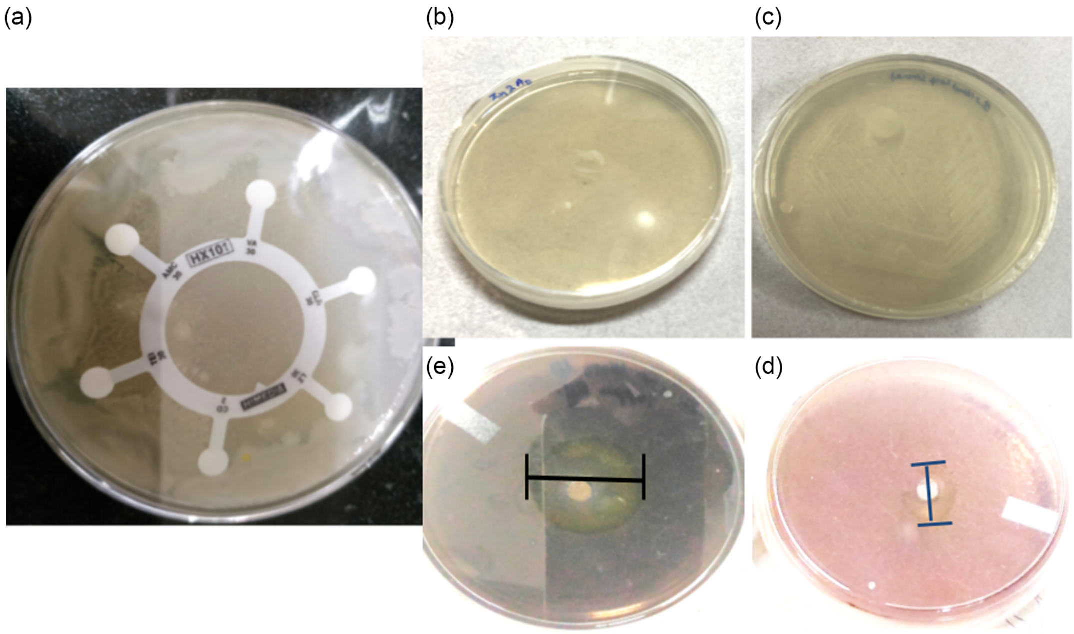


Figure 5
(a–e) represent the antibacterial activity tested against the bacterium



particularly in the region below 400 nm, signifies the nanoscale dimensions and elevated surface energy of the particles, both of which are pivotal for robust antibacterial performance. Nanoparticles with smaller dimensions, indicated by sharper and more intense absorption peaks, exhibit an enhanced surface-to-volume ratio, facilitating superior interaction with bacterial membranes and promoting the generation of reactive oxygen species (ROS). Furthermore, the SPR attributes, modulated by the concentration of bioactive compounds from *Tinospora cordifolia* extract, contribute to heightened stability and catalytic activity of the nanoparticles. Consequently, the optical properties, as elucidated through UV-Vis spectroscopy, serve as a reliable predictor of the ZnO nanoparticles' antibacterial potential, with heightened optical responses correlating with enhanced antimicrobial activity.

4. Discussion

The green synthesis of nanoparticles is an eco-friendly approach that offers significant advantages over traditional physical and chemical methods. The plant metabolites serve as natural reducing and stabilizing agents, eliminating the need for harmful chemicals and high-energy conditions. Compounds like flavonoids, alkaloids, tannins, terpenoids, and phenolics play a crucial role in the reduction and stabilization of nanoparticles which reduce metal ions, such as Zn^{2+} and Ag^{2+}, to their neutral forms through electron transfer, enabling the nucleation and growth of nanoparticles. At the same time, they stabilize the particles by forming a protective layer that prevents aggregation, with functional groups like hydroxyl (-OH) and carbonyl (-C=O) enhancing stability. Additionally, the type and concentration of plant extract influence the size and shape of the nanoparticles, as certain metabolites act as templates for their growth. This method is highly effective, cost-efficient, and scalable and produces nanoparticles with improved biological compatibility and enhanced antibacterial activity due to the phytochemicals on their surface. Green synthesis, therefore, not only supports sustainable practices but also provides nanoparticles with properties suitable for applications in medicine, environmental remediation, and catalysis.

Nanoparticles synthesized using *Tinospora cordifolia* leaf extract show strong antibacterial activity against multidrug-resistant bacteria, offering a potential alternative to conventional antibiotics. Their mechanism involves disrupting bacterial membranes, generating ROS that damage essential biomolecules, and leveraging their small size and large surface area for enhanced interaction and penetration. The antibacterial activity of *Tinospora cordifolia*-derived nanoparticles aligns with existing plant-based nanoparticle studies, showing broad-spectrum efficacy. Differences in activity may result from variations in size, shape, and surface charge, with smaller nanoparticles and certain shapes (e.g., spherical or rod-shaped) enhancing bacterial interaction. Phytochemicals on the nanoparticle surface enhance antimicrobial activity, offering added efficacy. Studies by Gul et al. [37] and Harun et al. [38] showed significant antibacterial effects of ZnO nanoparticles, even at low concentrations, against both MDR and non-MDR strains, highlighting their potential in combating resistant pathogens.

The optical properties of *Tinospora cordifolia*-synthesized nanoparticles, reflected in UV-Vis absorption peaks linked to SPR, reveal key information about their size and morphology. These traits confirm successful synthesis and influence their potential applications. These optical properties are vital for advanced applications like biosensing and antimicrobial drug delivery, where strong, tunable signals enhance detection sensitivity and support targeted therapeutic action. Their size and morphology-dependent optical properties enable stimulus-responsive release (e.g., light or heat), highlighting their versatility for therapeutic and diagnostic biomedical applications. The sustainable synthesis of ZnO nanoparticles with the help of *Tinospora cordifolia* leaf extract and their application is not without challenges. Variation in nanoparticle size is one of the main challenges encountered during the synthesis process. Variations arise mainly from inconsistent phytoactive metabolite concentrations, synthesis procedures, and environmental conditions. The variation in nanoparticle size can drastically influence antibacterial, optical, and other physiological properties, which significantly impacts the overall efficiency and reproducibility of the process. One major challenge is ensuring that synthesized nanoparticles remain stable over time. To ensure long-term stability in biological and environmental applications, it is often necessary to optimize synthesis parameters or add stabilizing agents, which may compromise the environmentally friendly aspect of the synthesis process. Furthermore, achieving precise control over the morphology and monodispersity of nanoparticles remains a technical hurdle that could affect their uniformity in applications such as biosensing or drug delivery. To overcome these limitations, we need to refine our synthesis protocols and improve our characterization techniques.

5. Conclusion

In conclusion, this study highlights the environmentally friendly and sustainable approach of using *Tinospora cordifolia* (Giloy) leaf extract for the successful green synthesis of nanoparticles. The nanoparticles showed significant antibacterial effectiveness against multidrug-resistant bacteria, emphasizing their potential as an alternative to traditional antimicrobial treatments. Additionally, an analysis of the nanoparticles' optical properties revealed promising potential for advanced applications in fields such as drug delivery and biosensing. These findings demonstrate how plants can mediate the production of nanoparticles to tackle modern challenges in nanotechnology and healthcare. Future research will concentrate on enhancing synthesis methods to facilitate scalable manufacturing while ensuring uniformity and optimizing parameters for improved nanoparticle performance and stability. A thorough investigation is necessary to uncover the specific mechanisms that govern the antibacterial efficacy of these nanoparticles, particularly their interaction with bacterial membranes and the induction of ROS. It is crucial to conduct in vivo studies to assess the therapeutic potential and safety of clinical applications. The antibacterial properties of these nanoparticles could be improved by integrating them into sophisticated drug delivery systems or hybrid nanostructures, providing innovative solutions to the increasing issue of multidrug-resistant infections.

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Ethical Statement

This study does not contain any studies with human or animal subjects performed by any of the authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest to this work.

Data Availability Statement

The data sharing is not applicable to this article as no new data were created or analyzed in this study.

Author Contribution Statement

Jyoti Jaglan: Conceptualization, Methodology, Writing – review & editing, Supervision. **Anshu Jaglan:** Methodology, Validation, Investigation, Writing – review & editing, Project administration. **Bajinder Singh:** Formal analysis, Resources. **Harsh Jaglan:** Software, Formal analysis, Resources, Data curation, Visualization. **Preeti Jaglan:** Validation, Investigation. **Savita Jaglan:** Investigation, Writing – original draft. **Monika Barala:** Conceptualization, Writing – original draft, Visualization, Project administration.

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