Facile Green Synthesis of Silver Doped ZnO Nanoparticles Using Tridax Procumbens Leaf Extract and their Evaluation of Antibacterial Activity

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ABSTRACT
Silver and zinc oxide are well known for both their antimicrobial and pro-healing properties. ZnO is a biocompatible and bio-safe material that possesses photo-oxidizing and photo-catalysis impacts on chemical and biological species. ZnO nanomaterials could interact chemically as well as physically in order to exhibit antibacterial activities. Chemical interactions of the ZnO nanomaterials with bacterial cells leads to the photo-induced production of reactive oxygenated species (ROS), formation of H2O2, and the release of Zn2+ ions. In contrast, physical interaction could show biocidal effects through cell envelope rupturing, cellular internalization, or mechanical damage. In this article, we present a green method using Tridax Procumbens leaf extract to synthesize Ag-doped ZnO nanoparticles (NPs) in order to explore the synergistic antibacterial properties of Ag and ZnO nanoparticles against certain gram-positive and gram-negative bacterial strains. The newly synthesized Ag-doped ZnO NPs were characterized by X-ray diffraction (XRD) in order to study the crystalline structure, composition, and purity. Transmission electron microscopy (TEM), Scanning electron microscopy (SEM) and Dynamic Light Scattering (DLS) techniques were used to study particle size, shape, and morphology. The XRD and UV studies confirmed the ZnO phase. The absorbance peak around 618 cm⁻¹ - 749 cm⁻¹ in the FTIR spectrum referred to the presence of silver. The surface morphological studies also supported the FTIR result. The synthesized sample exhibited enhanced antibacterial activity irrespective of all tested microorganisms than the standard antibiotic used. The maximum size distribution of particles is found to be around 60 nm from the DLS technique.

Keywords: Zinc Oxide nanoparticles, Green synthesis, Tridax Procumbens, Antibacterial Study, Gram-positive, and gram-negative bacteria

INTRODUCTION
Growing awareness of health and hygiene in recent years has emanated in an aggressive increase in the demand for antimicrobial agents as it is the breakthrough for the major issue of bacterial contamination. Various inorganic metal oxides have the potentiality for therapeutics, diagnostics, surgical device coatings, and nanomedicine applications. Among the metal oxides, Zinc Oxide (ZnO) is gaining attention in the last decade owing to its selective toxicity in biological systems [1]. As
the bandgap of ZnO is 3.37 eV and the excitation binding energy is 60 meV at room temperature, it is considered to be a promising material for optoelectronic devices, transparent conducting, piezoelectric, and photo-degradation (i.e. waste water treatment) applications [2-6]. Moreover, according to USFDA (US Food and Drug Administration), ZnO is considered as a non-toxic and low-cost material [7]. In addition, ZnO nanoparticles (NPs) have also been proposed as an antimicrobial preservative for wood and food products [8].

In recent days, not only the selection of material which is non-toxic and low cost is to be considered, but also the synthesis method having less toxicity, as well as cost-effectiveness, plays a vital role in the development of newer material suitable for health care technology.

Keeping aforesaid points in mind, in the present study silver doped ZnO nanoparticles (NPs) were synthesized using the green approach. It is well known that the green approach for the synthesis of NPs has the possibility of the least utilization of chemicals, low cost, and low energy requirement [9, 10]. Currently, the green synthesis of NPs using plants is emerging as a newer branch of nanotechnology due to its cost-effectiveness and eco-friendly alternative [11-15].

According to the literature survey, various research communities have synthesized ZnO based NPs from various plant sources and have been studied for its antimicrobial activity as reported in Table 1 [16-34]. In the present work, the leaf of Tridax procumbens has been used to prepare silver doped ZnO NPs (ZnO: Ag NPs). To the best of our knowledge, this is the first work on the synthesis of ZnO: Ag nanoparticles using Tridax procumbens leaf extract.

**Tridax procumbens**, a plant belonging to the daisy family is one of the most common plants

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Precursor</th>
<th>Leaf of Plant</th>
<th>Studied Microbes for Antimicrobial Activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Zinc Oxide</td>
<td>Green Tea</td>
<td><strong>S. aureus</strong></td>
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<td>2.</td>
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<td>Cucumis melo</td>
<td><strong>S. aureus</strong>, E. coli</td>
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<td>Antirrhinum</td>
<td><strong>S. aureus</strong>, <strong>B. subtilis</strong></td>
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<td><strong>Pseudomonas aeruginosa</strong></td>
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<td>6.</td>
<td>Zinc Nitrate</td>
<td>Isatis indigotica</td>
<td><strong>E. coli</strong>, <strong>S. aureus</strong>, <strong>Pseudomonas aeruginosa</strong></td>
<td>[21]</td>
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<tr>
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<td>Bungambia Nicotiana</td>
<td><strong>E. coli</strong>, <strong>S. aureus</strong></td>
<td>[22]</td>
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<td><strong>S. aureus</strong>, <strong>E. coli</strong></td>
<td>[27]</td>
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<td>[28]</td>
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<td>[30]</td>
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<td>[31]</td>
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<td><strong>E. coli</strong>, <strong>S. aureus</strong>, <strong>Pseudomonas Aeruginosa</strong>, <strong>Klebsiella pneumoniae</strong></td>
<td>[32]</td>
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<td><strong>E. coli</strong>, <strong>S. aureus</strong>, <strong>Serratia marcescens</strong>, <strong>Proteus mirabilis</strong></td>
<td>[33]</td>
</tr>
<tr>
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<td>Zinc Oxide</td>
<td>Chloranthes Scleas</td>
<td><strong>S. aureus</strong></td>
<td>[34]</td>
</tr>
</tbody>
</table>
used by rural and tribal communities in order to cure various health ailments. This plant habitats, waste places, roadsides, and hedges throughout India. Several reports from tribal areas in India state that the leaf extract of *Tridax procumbens* could be used to treat fresh wounds, stop bleeding, and also operate as a hair tonic [35-37]. In addition to this, *Tridax procumbens* is reported to have anti-inflammatory, immune modulator, anti-diabetic, hemostatic, antioxidant, hepatoprotective, antipyretic, and antimicrobial activity [38-40].

In the present investigation, the synthesis of ZnO: Ag NPs has a two-step procedure: First, the bio-reduction of silver nitrate by *Tridax procumbens* leaf extract using broth method and secondly, the preparation of ZnO: Ag NPs using a simple soft chemical route. These two methods have been proposed as cost-effective and the present study would be an environmentally friendly alternative to the chemical and physical methods. Hence, we have synthesized ZnO: Ag NPs via a green approach for antibacterial screening against certain gram-negative and gram-positive bacteria which will support the development of the disinfectant technology.

**MATERIALS AND METHODS**

**Green synthesis of silver (Ag) nanoparticles**

The silver nanoparticles were prepared using the broth method employed by Shankar et al. for the synthesis of Ag nanoparticles using Geranium leaf extract [41]. In the present study, fresh and healthy leaves of *Tridax procumbens* were collected from the Vellore District having latitude of 12.9165 N and 79.1325 E, during December 2019. The collected leaves were washed with Purified Milli-Q water in order to remove the unwanted impurities and dust particles and thereafter they were dried with filter paper to remove any drops of water from the leaves. 1 gm of the leaf was weighed and mixed with 100 ml of distilled water in a conical flask and boiled for 5 min. After cooling, the leaf extract was filtered with Whatman No.1 filter paper. To the 100 ml of the leaf extract, 1mM of Silver Nitrate (AgNO₃) was added and mixed nicely and was kept at room temperature for 5 minutes. The color change (greenish-yellow color changed to dark brown) was observed after 5 min of the addition of Silver Nitrate to the plant extract which indicates the formation of silver nanoparticles.

**Preparation of green synthesized ZnO: Ag NPs**

ZnO: Ag NPs were synthesized by a simple soft chemical route. 0.2 M of zinc acetate dihydrate [Zn(CH₃COO)₂·2H₂O] dissolved in 4:1 (i.e. 160 mL:40 mL) ratio of H₂O and the prepared plant extract (with silver NPs) is treated as the starting solution (200mL). A suitable amount of ammonium hydroxide pellets (NH₄OH) were added to maintain the pH level ≈ 8 of the starting solution. The obtained mixture is stirred for 2h under the temperature of 85 °C. The product is filtered, washed, and then calcined at 500 °C for 2h using Muffle Furnace (Model: Thermolyne benchtop muffle furnace (F48025-60)-duty 1200 °C). Subsequently, the final product is taken for further characterization.

**Characterization of the synthesized sample**

The crystalline structure of the green synthesized NPs was studied using the X-ray powder diffraction technique (PANalytical-PW 340/60 X’pert PRO) using CuKα radiation (λ = 0.15406 nm). The UV absorption spectrum is recorded using PerkinElmer (Lambda 750) UV-Vis-NIR spectrophotometer. Fourier transforms infrared (FTIR) spectrum was recorded using the PerkinElmer RX-I FTIR spectrophotometer employing the KBr technique. The surface morphology of the synthesized ZnO: Ag NPs was observed using a scanning electron microscope (Carl Zeiss Ultra 55 FE-SEM). The microstructure of the Phyto-mediated Ag-doped ZnO NPs was analyzed using a transmission electron microscope (TEM, Hitachi H-7100). The size of the synthesized particles was measured using a particle size analyzer (Micromeritics, Nano Plus model).

**Antimicrobial Assay**

The antimicrobial activity of green synthesized ZnO: Ag NPs was tested against six different bacteria using the agar well diffusion method. For bacterial growth, Mueller Hinton Broth is used as a nutrient agar medium. This agar medium contains beef extract, peptone, sodium chloride, and yeast. Later on, the prepared medium was sterilized in an autoclave at 120 °C for 20 min, and subsequently, it was poured into a sterile petri dish and was allowed to solidify in a laminar airflow chamber. After solidification, by using a sterile cotton swab, fresh bacterial culture was spread over the plate...
using a spread plate technique. Three wells were bored into the plates using a sterile cork borer of 5 mm in diameter one each for control, for standard antibiotic Nitrofurantoin, and for stock solution having the synthesized sample (300 µg/mL). After loading the wells, the bacterial plates were incubated at 37 °C for 24 hr. Later on, the plates were monitored for the clear inhibition zone around the well. The diameter of the inhibition zone was measured in mm. In the present study, three-gram positive bacterial cultures namely Staphylococcus aureus, Streptococcus pyogenes, and Bacillus subtilis, and three-gram negative bacterial cultures namely Escherichia coli, Klebsiella pneumonia, and Pseudomonas aeruginosa were used as test microbes.

RESULTS AND DISCUSSION

Structural Studies

The characterization of the crystalline material and the evaluation of the crystal structure, crystal orientation, and crystal defects can be studied using XRD analytical technique. The XRD pattern of green synthesized ZnO:Ag NPs is shown in Fig. 1a. This XRD pattern exhibits the diffraction peaks at = 32º, 34º, 36º, 47º, 56º, 63º, 66º and 68º corresponding to (100), (002), (101), (102), (110), (103), (200) and (112), respectively. The observed peaks indicate the wurtzite crystalline structure and hexagonal phase of ZnO which are relevant to those in the ZnO standard powder diffraction file JCPDS card no: 00-036-1451.

From this XRD pattern, it is obvious that there is no significant peak for Ag related phases or any considerable shift in the location of the diffraction peaks as compared with standard ZnO. This implies that the lattice structure of the ZnO crystal has not significantly changed by the incorporation of biosynthesized silver nanoparticles into the ZnO lattice.

Using the following Debye-Scherrer’s formula \[ D = \frac{k\lambda}{\beta \cos \theta} \] the crystallite size (D) of the NPs was calculated:

Where k is a constant equal to 0.90, λ is the wavelength of the incident X-rays, β is the full width at half maximum (FWHM) in radian, and θ is the Bragg’s angle in radian. The calculated value of the crystallite size is found to be 50 nm. This is similar to the previous literature where ZnO NPs synthesized using Costus pictus leaf extract [44] is having a crystallite size of 29.11 nm, and the work related to Mentha pulegium leaf extract mediated synthesis of ZnO NPs reports a crystallite size of 44.94 nm [29]. Another literature where ZnO NPs have been synthesized using Anisochilus carnosus...
leaf extract also indicated similar lattice structures obtained in the XRD pattern of the ZnO NPs and it reports various sizes which include 56.14, 49.55, and 38.59 nm, for 30, 40, and 50 ml of extract addition, respectively [15]. Hence, it is believed that the addition of phytochemicals plays a vital role in enhancing the crystalline nature of ZnO.

In addition to these, the crystallite size was also calculated from the Williamson-Hall (W-H) plot. This helps to evaluate the size and strain component of the peak broadening simultaneously unlike the conventional Debye-Scherrer’s equation. Furthermore, it enables $h\ k\ l$-dependent broadening and crystallite shape prediction (Transformation from Conducting Ferromagnetic to diamagnetic insulator). W-H equation [45-48] is given below:

$$\beta \cos \theta = \frac{k\lambda}{D} + \left(4\varepsilon \sin \theta \right)$$  \hspace{1cm} (2)

Where the notations $\beta$, $\theta$, $\lambda$, $D$, and $k$ have their usual meaning as explained in the previous section and $\varepsilon$ refers to induced lattice strain. From the W-H plot (Fig. 1b), the estimated values of crystallite size and lattice strain are found to be 108 nm and $\sim 0.1$ % respectively. The crystallite size estimated from the W-H plot (108 nm) is found to be more than that of the D-S (50 nm) method which emphasizes the role of the induced lattice strain in the prepared ZnO: Ag nanoparticles. Such a difference in the value of crystallite size estimated from these two methods has been reported in the literature [49, 50].

**Optical Studies**

Fig. 2a shows the absorption spectrum of the biosynthesized ZnO: Ag NPs recorded in the range of 300–1000 nm. The absorption peak obtained at 372 nm is due to their large excitation binding energy of ZnO at room temperature [51]. This absorption edge peak at 372 nm implies the blue-shift of the synthesized ZnO NPs compared with its bulk counterpart (377 nm) which is owing to it to the quantum confinement effect [52, 53]. A similar shift was obtained in previous literature in which ZnO NPs have been synthesized using Laurus nobilis [54], Solanum torvum [55], Ixora coccinea [56], and Parthenium hysterophorus [8], leaf extracts with the absorption edge in the range of 340–375 nm, confirming the narrow particle size distribution [57]. In the present study, due to this quantum confinement effect, the obtained band gap (3.33 eV) is higher as compared with the bulk ZnO (3.29 eV).

The bandgap energy of ZnO nanoparticles is calculated using Tauc’s plot using the formula given below [58-60]:

$$\alpha = \frac{1}{d} A \ \text{(cm$^{-1}$)}$$  \hspace{1cm} (3)

Where $d$, is the sample cell thickness, (cm) $A$ is the absorbance (arb. unit) $\alpha$ is the absorbance coefficient, (cm$^{-1}$)

$$\alpha h \nu = (h \nu - E_g)^n$$  \hspace{1cm} (4)

where $E_g$ is the bandgap of the material, (eV) $h$ is Planks Constant ($6.626\times10^{-34}$ Js), $\nu$ is the frequency given by ($c/\lambda$), (Hz) $c$ is the velocity of light ($3\times10^8$ m/s)
and $n$ is the exponent which depends on the type of the bandgap.

The bandgap energy calculated using Tauc’s plot (Fig. 2b) is found to be 3.3 eV.

**FTIR studies**

The FTIR spectrum of ZnO: Ag nanoparticles is shown in Fig. 3. These particles indicated a broad peak at 3397 cm$^{-1}$ which could be attributed to the characteristic absorption of the hydroxyl group and the peak at 479 cm$^{-1}$ is formed due to the absorption of Zn-O bonds [61]. The peak near 2927 cm$^{-1}$ is assigned to the alkyne group present in the phytoconstituents of the *Tridax procumbens* leaf extract [62]. The peak around 600 cm$^{-1}$ to 800 cm$^{-1}$ refers to silver [63]. Hence, the observed smaller peaks near 618 cm$^{-1}$ to 749 cm$^{-1}$, confirm the incorporation of silver NPs into the ZnO lattice. Furthermore, the peak around 700 cm$^{-1}$ is responsible for the presence of emodin [64] which is a bioactive compound derived from the *Tridax procumbens* leaf extract in the sample. The other peaks around 1300-1400 cm$^{-1}$ are responsible for the presence of NO$_2$ symmetric stretching which is also due to the presence of leaf extract in the starting solution. Hence, the observed FTIR results indicate that the green synthesized silver nanoparticles are incorporated into the ZnO lattice and are in agreement with the literature. In addition, certain other phytochemicals of the *Tridax procumbens* leaf extract were also identified from the spectrum.

**Dynamic Light Scattering (DLS) Analysis**

DLS technique is widely used in order to identify the particle size distribution of the nanoparticles. Fig. 4 indicates that the distribution of particle size for the phytomediated silver doped ZnO NPs is from 40 to 120 nm with the maximum size distribution is around 60 nm. From this result, it is assumed that the phytochemicals of the leaf extract present in the starting solution influence the particle size of the ZnO NPs to be in the nano range.

**SEM and TEM Analyses**

The SEM image of the ZnO: Ag NPs is shown in Fig. 5. This SEM micrograph shows that most of the nanoparticles are in a hexagon shape which is the basic structure of ZnO and spherical shaped nanoparticles are also observed. In addition to this, certain nano-stick-like structures are also observed (inset of Fig. 5) indicating that Ag enters into the ZnO lattice and influences the morphology of the synthesized samples [30]. Moreover, these ZnO: Ag NPs exhibit uniform morphology with certain voids. These voids play a vital role in exhibiting enhanced antimicrobial activity [65] as evidenced by the forthcoming section (Antimicrobial analysis).

The transmission electron micrograph of the phytomediated ZnO: Ag NPs is shown in Fig. 6. This image reveals that most of the nanoparticles are spherical and their diameter is found to be around 18 to 22 nm. The particles are also shown to be quasi-spherical and hexagonal shaped. In addition to this, certain nanoparticles exhibit a nano-stick-like structure as evidenced by the inset of Fig. 6. It is in good agreement with the surface morphology recorded by the SEM technique.

It is well known that SEM is used for
imaging the surface of the nanoparticle on a submicroscopic scale. In contrast, the use of TEM is to image the internal structure of the particle on a nanometer scale. Hence, the particles appear ~3% larger in the SEM than in the TEM microscope. In the present study, the average particle size is observed to be ~20 nm from the TEM image whereas the SEM image is ~55 nm. The formation of these smaller sized (~20 nm) nanoparticles are the ones responsible for boosting the antibacterial activity of the green synthesized ZnO: Ag NPs.

**Antibacterial Studies**

The antibacterial activity of Ag-doped ZnO NPs was examined for gram-positive bacteria like *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Bacillus subtilis*, and gram-negative bacteria like *Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*, and their impacts are shown in Fig 7 a and b, respectively. From these figures, it is obvious that the green synthesized Ag-doped ZnO NPs exhibit a remarkable antibacterial activity against all six foodborne pathogens as denoted by the zone diameter of inhibition in mm. Fig. 8a shows the inhibitory efficacy of the green synthesized ZnO: Ag NPs against gram-positive bacteria over the standard antibiotic used; whereas Fig. 8b shows for gram-negative bacteria over the standard antibiotic used. Although the...
Fig. 7. a: Photos of agar plates inoculated with gram positive bacteria a) *Staphylococcus aureus* b) *Streptococcus pyogenes* and c) *Bacillus subtilis* together with green synthesized ZnO:Ag NPs
b: Photos of agar plates inoculated with gram negative bacteria a) *Escherichia coli*, b) *Klebsiella pneumonia* and c) *Pseudomonas aeruginosa* together with green synthesized ZnO:Ag NPs
Fig. 8. a. The inhibitory efficacy of green synthesized ZnO:Ag NPs against gram positive bacteria
b. The inhibitory efficacy of green synthesized ZnO:Ag NPs against gram negative bacteria
synthesized sample shows enhanced antibacterial activity against all the tested bacteria, it exhibits comparatively higher efficiency against gram-positive bacteria than gram-negative bacteria which could be understood from the bar diagram represented in Fig. 8 a and b. The present findings are in good agreement with the reports given by various researchers working with different leaf extract based ZnO NPs [66, 67] and their reports suggest that this phenomenon may be attributed to the difference in the cell wall structures of the gram-negative and positive bacteria. As the gram-positive bacteria have a less complicated cell wall structure compared to gram-negative bacteria it is easy for the toxicants (here it is ZnO: Ag NPs) to access the sites of action for rupturing the cell structure which leads to death of it.

Some researchers observed lesser antibacterial activity for green synthesized ZnO based NPs when compared to a standard tablet used [15, 68-71]. But contrary to their results, in the present study, we observed higher antibacterial activity for the green synthesized ZnO: Ag NPs as compared to the standard antibiotic used (Nitrofurantoin) which may be due to the smaller/reduced particle sizes (∼20 nm) of the synthesized nanoparticles which provides more surface area to interact with microbes, resulting in enhanced antimicrobial activity. It is well known that the antibacterial efficacy of ZnO NPs was inversely proportional to the size of nanoparticles [72]. From the literature survey, it is inferred that the green synthesized ZnO NPs exhibit better antibacterial activity when compared with the chemically synthesized ZnO NPs and are even with simple/bare plant extracts [68,73, 74].

As it is already well known, the antibacterial mechanism of Ag and ZnO NPs is having the following sequence: i) release of Ag⁺ and Zn²⁺ ions, ii) direct attachment of these ions onto cell surfaces, and iii) production of reactive oxygen species (ROS) to attack the bacterial cell as consequence apoptosis of it [75-77].

In addition to these, the enhanced antibacterial activity of the synthesized sample is due to the presence of certain phytochemicals of the Tridax procumbens leaf extract in it. Some of the research groups [78-80] subjected the Tridax procumbens leaf extract for qualitative phytochemical screening to identify the presence of chemical constituents like Steroids, Saponin, Alkaloids, Phenol, proteins, emodins, amino acids, etc., Among these, emodin is found to be a highly bioactive compound. Based on its in-vivo studies it is proved to have therapeutic potential like Strong Anti-inflammatory, antioxidant, cardiovascular, CNS, Antineoplastic agent, metabolic, hepato-protective, respiratory, anti-microbial, laxative, and immune-modulator. From this literature survey, it is believed that the presence of emodin in our synthesized sample will also play a role in enhancing the antibacterial efficacy. The presence of emodin could be identified from the FTIR spectrum Sec. 3.3. Thus in this case aforesaid factors are combined in order to work together for damaging the bacteria and as a consequence, enhancing the antibacterial efficacy of the synthesized sample.

CONCLUSION

To summarize, Ag-doped ZnO NPs were synthesized by an ecofriendly green approach using Tridax procumbens leaf extract. The XRD pattern illustrated that the synthesized sample has hexagonal structure of ZnO. The crystallite size calculated from the Scherrer method was found to be 50 nm whereas using Williamson-Hall method was found to be 108 nm along with the lattice strain 0.81×10⁻³. FTIR spectrum and the surface morphological (SEM & TEM) studies confirmed the presence of Ag into the ZnO lattice. The excellent synergic antibacterial properties of the newly synthesized Ag-doped ZnO NPs was observed for gram-positive and gram-negative bacteria. It was observed that Ag-doped ZnO NPs exhibited comparatively higher antibacterial activity when compared to the standard antibiotic Nitrofurantoin; this was due to the smaller particle size with the presence of the phytochemicals of Tridax procumbens leaf, mainly Emodin. The presence of emodin was also confirmed by the FTIR studies. This is an advancement over traditional treatment methods as maximum bacterial strains have developed multiple antibiotic resistances toward commonly used antibiotic drugs.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.
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