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Green synthesis of zinc oxide nanoparticles (ZnO-NPs) using *Ailanthus altissima* fruit extracts and antibacterial activity

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ABSTRACT

The green synthesis of nanoparticles using plants fruits aqueous extracts have been prepared for different applications. Recently, the green synthesis of zinc oxide nanoparticles have been a keen interest amongst researchers and scientist due to its simplicity, eco-friendliness, non-toxic, inexpensive and potential to perform as antibacterial agent. Thus, in this current work, the synthesis of zinc oxide nanoparticles using *Ailanthus altissima* fruits aqueous extract is reported. The green synthesized zinc oxide nanoparticles were characterized using scanning electron microscope (SEM), UV-Vis absorption spectroscopy and X-ray diffraction (XRD). The average size of the nanoparticles prepared was found to be ranging from 5 to 18 nm. The FT-IR analysis suggests that the obtained ZnO-NPs have been stabilized through the interactions of flavonoids, and phenolic compounds in the fruits extract. Synthesized ZnO-NPs reveals antibacterial activity against two bacterial strains, *Escherichia coli* and *Staphylococcus aureus*. It is concluded that the ZnO-NPs have high efficient antibacterial activity and considered as a potential additive to substitute toxic chemical and physical antibacterial materials.

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Capsule Summary: Green synthesis of ZnO-NPs using *Ailanthus altissima* fruits aqueous extract and prepared ZnO-NPs showed promising antibacterial activity against *E. coli* and *S. aureus*.

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INTRODUCTION

Zinc oxide nanoparticles (ZnO-NPs) with different morphological structures as antibacterial activity against bacteria micro fungus and virus was developed using various synthetic routes such as wet chemical (Narkiewicz et al., 2008), microemulsion (Yildirim, and Durucan, 2010), hydrothermal (Shao-Hwa et al., 2010), solvothermal

(Shamhari et al., 2018), precipitation and co-precipitation route (Ghorbani et al., 2015; Katiyar et al., 2018), surfactant-assisted precipitation routes (Mahamuni et al., 2018), polyol chemistry (Mohan and Renjanadevi, 2016), conventional methods using surfactant (Mohan and Renjanadevi, 2016), Microwave-assisted combustion (Kooti and Sedeh, 2013), sonochemical method (Khanna et al., 2012), and sol-gel method (Hasnidawani et al., 2016; Chung et al., 2015; Al Abdullh et al., 2017). These methods have many

disadvantages due to the difficulty of scale up the process, separation and purification of nanoparticles from the micro emulsions and energy requirements. Developing facile and green methods for synthesizing zinc oxide nanoparticles are of importance and still a challenge for materials researchers. According to the literature surveys, plants extracts have been suggested as valuable alternatives to chemical methods for synthesis of zinc oxide nanoparticles.

Recent research reported the synthesis of zinc oxide nanoparticles (ZnO-NPs) using different plant extracts such as *Tabernaemontana divaricata* green leaf extract (Raja et al., 2018), flower extract of *Nyctanthes arbor-tristis* (Jamdagni et al., 2018), *Azadirachta indica* leaf extract (Bhuyan et al., 2015), *Laurus nobilis* L. leaves aqueous extract (Fakharij et al., 2019), *Aloe barbadensis miller* leaf extract (Sangeetha et al., 2011), *Cassia alata* fresh leaves (Happy et al., 2019), leaf extracts of the *Costus pictus* D. Don medicinal plant (Suresh et al., 2018), aqueous extracts of *Aloe vera* gel/leaf and *Hibiscus sabdariffa* leaf (Mahendiran et al., 2017), *Hibiscus rosa-sinensis* leaf extract (Divya et al., 2013), *Ixora coccinea* leaf extract (Yedurkar et al., 2016), cherry extract (Mohammadi and Ghasemi, 2018), *Sesamum indicum* L. (Manokari et al., 2019), *Moringa oleifera* leaves (Matinise et al., 2017; Pal et al., 2018), *Pongamia pinnata* leaves (Sundrarajan et al., 2015), *Anisochilus carnosus* leaves (Anbuvaran et al., 2015), *Moringa Oleifera* seed (Rajeswari et al., 2018), *Ocimum basilicum* L. var. *purpurascens* Benth.-Lamiaceae leaves (Salam et al., 2014), *Plectranthus amboinicus* leaves (Fu and Fu, 2015), *Abutilon indicum* leaves (Ijaz et al., 2017), *Juglans regia* leaves (Asemani, and Anarjan, 2019), *Syzygium alternifolium* flowers (Yugandhar et al., 2017), *Atalantia monophylla* leaf extracts (Vijayakumar et al., 2018), leaf extract of *Glycosmis pentaphylla* (Vijayakumar et al., 2018), leaf extract of *Mentha pulegium* (Rad et al., 2019), *Lycopersicon esculentum* (tomato), *Citrus sinensis* (orange), *Citrus paradisi* (grapefruit) and *Citrus aurantifolia* (lemon) peels (Nava et al., 2017), plant extract of flower *Matricaria chamomilla* L., *olea europaea* leaves and *Lycopersicon esculentum* M. fruits (Ogunyemi et al., 2019), fruits of *Ananas Comosus* (Ahmad et al., 2019), *Ixora Coccinea* leaves (Yedurkar et al., 2016), *Ipomoea pescaprae* leaves (Venkatesan et al., 2017), *Corymbia citriodora* leaves (Zheng et al., 2015), tomato extract (Sutradhar and Saha, 2017), *Moringa Oleifera* Leaf Extract (Pal et al., 2018), *Boswellia ovalifoliolata* stem bark-extract (Supraja et al., 2016), root extract of *Zingiber officinale* (Raj and Jayalakshmy, 2015), extracts of *Allium sativum*, *Rosmarinus officinalis* and *Ocimum basilicum* (Stan et al., 2016), *trifolium pratense* flower extract (Renata and Długaszewska, 2015), *Punica granatum* fruit peels extract (Sukr et al., 2019), *Capsicum annum* fruit extract (Lalithamba et al., 2018), *Gooseberry* fruits extracts (Vennila, and Jesurani, 2017) and *Garcinia mangostana* fruit pericarp (Aminuzzaman et al., 2018).

In the present investigation, *Ailanthus altissima* fruits aqueous extract was used for the synthesis zinc oxide nanoparticles (ZnO-NPs), which were characterized by advanced techniques and antibacterial activity was also

evaluated. The fruits of *Ailanthus altissima* grow in clusters, contain hundreds of seeds (Fig. 1A).

MATERIAL AND METHODS

Chemical and reagents

Zinc nitrate hexahydrate (Purum $\geq 98\%$ Zn (NO₃)₂·6H₂O) was purchased from Sigma-Aldrich, Germany). De-ionized water was used in all experimental work. *Ailanthus altissima* fruits were collected from trees at Royal scientific Society, Jordan and washed with water to remove dust particles. Fruits were dried at ambient temperature for 7 days and then ground to obtain fine powder. The powder was then sieved with 350 mesh sieve.

Preparation of *Ailanthus altissima* fruits aqueous extract

Ailanthus altissima fruits powder (10 g) were mixed with 1L de-ionized water and boiled for 10 min at 80 °C to obtain reddish aqueous extract and then left to cool at ambient temperature. Afterward, aqueous extract was obtained by filtration on Whitman's filter paper and kept for our research work.

Synthesis of zinc oxide nanoparticles (ZnONPs)

Zinc nitrate hexahydrate [(ZnNO₃)₂·6H₂O] 10 g was dissolved in 200 mL de-ionized water under stirring with magnetic bar at ambient temperature (27 °C). Afterward, an aqueous extract of *Ailanthus altissima* fruits was added drop by drop to zinc solution till the solution started changing from colorless color to reddish brown and the formation of suspended particles. The mixture was left overnight and filters to obtain the suspended particles, which dried in an oven at 80°C for 4h. The powder obtained was subjected to analysis by X-ray diffraction (XRD) and scanning electron microscopy (SEM) (Fig. 1B).

Characterization techniques

Fourier infrared spectroscopy (FT-IR; IR Prestige-21, Shimadzu) was used to identify the different chemical functional groups present in the *Ailanthus altissima* fruits. FT-IR analyses also used to determine the functional groups are responsible for the reduction of zinc nitrate to zinc oxide nanoparticles. The analysis was carried out using KBr and the spectral range varying from 4,000 to 400 cm⁻¹. X-ray diffractometer, XRD-6000 (Shimadzu, Japan) equipped with Cu K α radiation source using Ni as filter at a setting of 30 kV/30mA. All XRD data were collected under the experimental conditions in the angular range $3^\circ \leq 2\theta \leq 50^\circ$. Scanning electron microscopy (SEM) analysis of synthesized ZnO nanoparticles was done by S-4500 SEM machine (Hitachi, Japan). Formation and stability of ZnO-NPs in sterile distilled water is confirmed using UV-vis spectrophotometer in a range of wavelength from 200 to 700 nm.



Fig. 1A: *Ailanthus altissima* fruits used for the green synthesis of ZnO-NPs



Fig. 1B: Schematic presentation of ZnO-NPs synthesis using *Ailanthus altissima* fruits extracts, analysis and antimicrobial activity

Antibacterial activity

Green synthesized ZnO-NPs using *Ailanthus altissima* fruits aqueous extract were studied for their antimicrobial activity against pathogenic bacteria by disc diffusion method; it was observed that ZnO-NPs have antibacterial activities at concentration of 2 $\mu\text{g}/\text{disc}$. Chloromphenical was used as a control antimicrobial agent.

RESULTS AND DISCUSSION

Optical properties of ZnO-NPs

The color of the reaction mixture changed from colorless to pale brown during reaction and from brown to white on heating to a temperature of 450°C using muffle furnace for 2h. Optical properties of ZnO-NPs using UV-vis spectrum

were analyzed by measuring the UV-vis spectrum (Fig. 2), which clearly indicated that the intense characteristic absorption peaks were between 360 to 369 nm which confirmed the formation of ZnO-NPs.

FTIR analysis

FTIR measurement was carried out to identify the possible biomolecules in *Ailanthus altissima* fruits extract responsible for the reduction and capping processes leading to an efficient stabilization of ZnO-NPs. The FTIR spectrum showed the presence of bonds due to -OH stretching frequency around 3424 cm^{-1} . However, peaks at 2959 cm^{-1} and 2865 cm^{-1} are attributed to the asymmetric and symmetric stretching vibrations of -CH₂ group respectively. The peak at 1646 cm^{-1} results from the stretching bands of C=O functional groups.

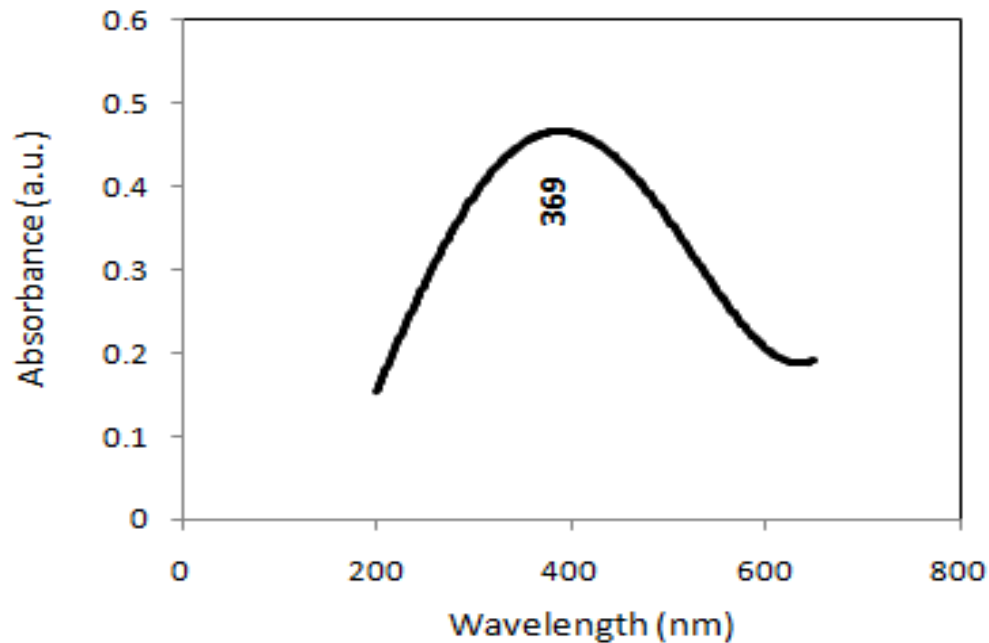


Fig. 2: UV-vis spectrum of ZnO-NPs synthesized via green route

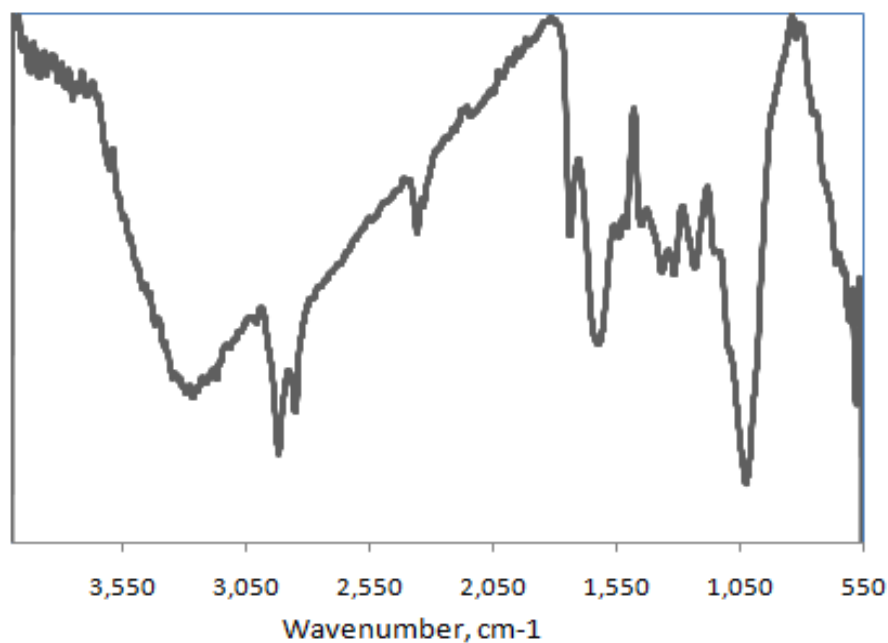


Fig. 3: FT-IR spectrum of *Ailanthus altissima* fruits extract (Y-axis = Transmittance, %)

The peak at 1758 cm^{-1} referred to the presence of stretching vibrations of non-ionic carboxylic acids or acid esters present in *A. altissima* fruits. The peak at 1367 cm^{-1} indicated the presence of C-O-H bending mode. The peak at around 1387 cm^{-1} present in ZnO signified amide band of the random coil of protein. The peak at 612 cm^{-1} indicates the stretching vibrations of ZnO-NPs. The region between

500 and 600 cm^{-1} is assigned for metal-oxygen bond. In addition to the bands of the biomolecules used as reducing and stabilization agents. The absorption peak at 584 cm^{-1} indicates the presence of ZnO-NPs (Fig. 3).

X-ray diffraction analysis

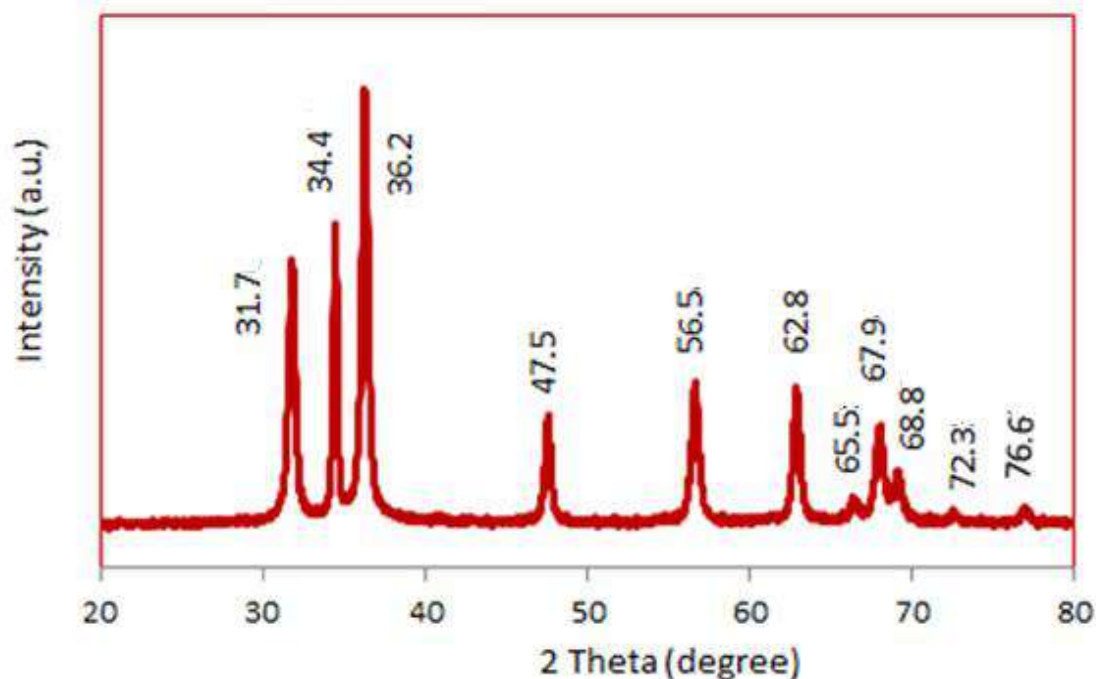


Fig. 4: XRD pattern of the ZnO-NPs synthesized using *Ailanthus altissima* fruits aqueous extract

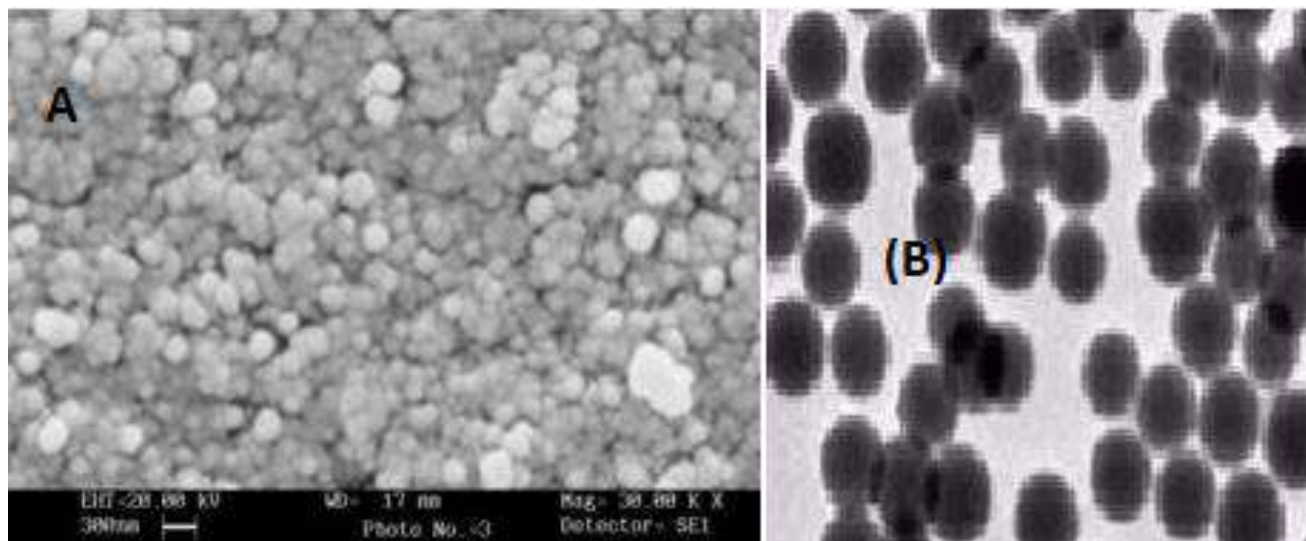


Fig. 5: Scanning electron microscopy (A-SEM) and Transmission electron microscopy (B-TEM) ZnO-NPs synthesized using *Ailanthus altissima* fruits aqueous extract

The X-ray diffraction (XRD) pattern of synthesized ZnO-NPs is illustrated in Fig. 4. The XRD pattern revealed the orientation and crystalline nature of zinc oxide nanoparticles. The peak position with 2θ values of 31.7° , 34.4° , 36.2° , 47.5° , 56.6° , 62.8° , 65.5° , 67.9° , 68.8° , 72.3° and 76.7° are indexed as (100), (002), (101), (102), (110),

(103), (200), (112), (201) and (202) planes, which are in good agreement with those of powder ZnO obtained from the International Center of Diffraction Data card (JCPDS-36-1451) confirming the formation of a crystalline monoclinic structure. No extra diffraction peaks of other phases are detected, indicating the phase purity of ZnO-NPs. The

average crystallite size of the D of synthesized nanoparticles was calculated using the well-known Scherrer formula as shown in Eq. 1.

$$D = 0.9\lambda/\beta\cos\theta \quad (1)$$

Where, λ is the wavelength of X-ray source (Cu-K α line-0.1541 nm), β is the full width at half maximum (FWHM) in radians and θ is Bragg's diffraction angle. The calculated value of D was 24 nm.

SEM and TEM analysis of ZnO-NPs

The morphology of green synthesized ZnO-NPs during the synthesis process could be observed visually. The addition of *Ailanthus altissima* fruits aqueous extract to colourless zinc nitrate hexahydrate solution, changed to brown in about 10min. The completion of ZnO-NPs formation, takes 2h under magnetic bar stirring and at temperature 70-80°C for 2h. The formation of ZnO-NPs was confirmed by UV-vis spectrum (Fig. 1) and morphology of synthesized ZnO-NPs as observed by SEM and TEM is presented in Fig. 5. The formed ZnO-NPs have spherical shape. The particle size of the synthesized ZnO-NPs by this green method showed the particle size range from 5-40 nm and with an average size of 20 nm.

Antibacterial activity of ZnO-NPs

Synthesized ZnO-NPs were tested by disk diffusion method using selected organisms such as Gram-negative bacteria *E. coli*, and Gram-positive bacteria *S. aureus*. As the concentration of ZnO-NPs increases, the zone of inhibition

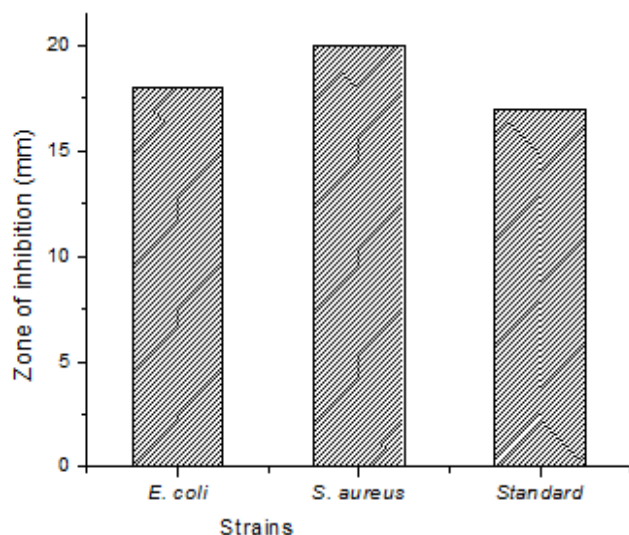


Fig. 5: Antimicrobial activity of the ZnO-NPs synthesized using *Ailanthus altissima* fruits aqueous extract

also increased against the bacteria strain. Zone of inhibition values determined for the treated ZnO-NPs (0.5mg/ml) were shown in Table 1. Zinc oxide nanoparticles have a significant growth inhibition effect against *S. aureus* and *E. coli* bacteria. This effect is resulted from high surface area of ZnO-NPs and their small particle size 12 nm. These findings are in line with previous studies that ZnO fabricated via green route, were highly active antimicrobial agents, i.e., *Acalypha fruticosa* L. leaf extract mediated synthesis of ZnO-NPs was performed and antimicrobial activities were tested against *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* and ZnO prepared via green route showed excellent antimicrobial activity (Vijayakumar et al., 2019). Similarly, *Medicago sativa* L. extract was used for the synthesis of ZnO-NPs and antimicrobial activity was evaluated against *Staphylococcus epidermidis* ATCC49461, *Lactococcus lactis* ATCC49032 and *Lactobacillus casei* ATCC334 and yeast (*Candida albicans* ATCC10231 and *Saccharomyces cerevisiae* MG012794). It was observed that the ZnO-NPs fabricated via green route were highly active against panel of strains (Król et al., 2019). Also, ZnO nanoparticles prepared via green route using *Raphanus sativus* root extract showed excellent on antimicrobial activity against MDR strain for wound healing applications (Kiran Kumar et al., 2019). Hence, the ZnO prepared via green route using *Ailanthus altissima* fruits aqueous extract could possibly be used as antimicrobial agent. In view of negative impact of conventional methods for the synthesis of nanoparticle, green synthesis is best alternative for the synthesis of NPs (Balwe et al., 2017; da Silva et al., 2019; Kumar Sur et al., 2018; Suresh et al., 2018).

CONCLUSIONS

Green synthesis of ZnO-NPs using *Ailanthus altissima* fruits aqueous extract was investigated in present investigation. The main advantage of this synthesis is its simplicity, cost-effective synthetic route and large scale production of ZnO-NPs. The formation of ZnO-NPs was confirmed by SEM, TEM and XRD analysis. The synthesized nanoparticles were highly active as antibacterial agent against pathogenic bacteria like *E. coli* and *S. aureus* at a very low concentration. It is concluded based on results, that green synthesis using *Ailanthus altissima* fruits aqueous extract is an efficient synthesis route for ZnO-NPs at nano-scale, which can also be extended for the synthesis of other metal oxide NPs.

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