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Original article

Green synthesis of ZnO nanoparticles for antimicrobial and vegetative growth applications: A novel approach for advancing efficient high quality health care to human wellbeing

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ABSTRACT

The present work aims to synthesize zinc oxide (ZnO) nanoparticles via green approaches using leaf extract of *Parthenium hysterophorus*. UV–vis and FT-IR tests confirmed the existence of biomolecules, active materials, and metal oxides. The X-ray diffraction structural study exposes the ZnO nanoparticles formation with hexagonal phase structures. SEM and TEM analysis reveal surface morphologies of ZnO nanoparticles and most of them are spherical with a size range of 10 nm. ZnO nanoparticles were revealed strong antimicrobial activity against both bacterial and fungal strains. The germination of seeds and vegetative growth of *Sesamum indicum* has been greatly improved.

1. Introduction

Nanotechnology is a multidisciplinary scientific domain and has been used in various science fields, including chemical, physical, biological, pharmaceutical and material science (Porter and Youtie, 2009; Govindarajan et al., 2016a,b; Govindarajan, M. and Benelli, 2016, 2017; Balalakshmi et al., 2017; Divya et al., 2018; Fahimmunisha et al., 2020). The promising application of nanotechnology unlocked up a new scope and perspective in agriculture. The relatively small size, high surface to volume ratio and characteristics optical properties of nanomaterials find the application from plant protection to nutrition and management practices in the farm (Shang et al., 2019). The perceptive of nanotechnology provides a new precision to agriculture with particular reference to fertilizer. The effects and efficiency of nanoparticle uptake on growth and metabolic activities may vary between the plants (Rastogi et al., 2017). The uptake concentration of nanoparticle influences the germination process and plant growth. Deficiency of zinc (Zn) is one of the major micronutrient problems affecting crop production, mostly calcium carbonate-rich alkaline soils (Takkar and Walker, 1993). The calcium carbonate abundant soils and alkaline pH may reduce both the obtainability and solubility of Zinc to the crops (Alloway, 2009; Rashid and Ryan, 2004). The Zn fertilizers such as zinc oxide (ZnO) and zinc sulphate were used to compensate the Zn deficiency in soils (Mortvedt, 1992) but were limited to their applications due to Zn non-availability to the plants. Apart, the application of chemical fertilizer leads to adverse effects on livestock, beneficial soil microorganisms and finally reduces soil fertility. In order to combat this problem, more effective and non-persistent fertilizer such as controlled release formulation is therefore required. ZnO nanoparticles

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as a source of Zn provide more bio-available form, Zn dissolution, bioavailability in soil, and plants to enhance its further development.

For the synthesis of nanoparticles, many physical and chemical methods are available which use toxic chemicals as a major constituent (Kumar et al., 2013; Suganya et al., 2017; Ishwarya et al., 2017a,b; Thaya et al., 2018; Karthika et al., 2020; Kiriyanthan et al., 2020; Sebastiammal et al., 2020). So the need of the hour is to use greener, environmentally benign and eco-friendly routes for the synthesis of metallic nanoparticles (Karthika et al., 2017). Among all, the synthesis of metallic nanoparticles using plants or plant residue is a low-cost, eco-friendly and energy-efficient method followed so far (Veerakumar et al., 2014; El Shafey, 2020; Vijayakumar et al., 2020; Vinotha et al., 2020). The chemical synthesis of ZnO nanoparticles has been reported earlier (Meruvu et al., 2011) but green synthesis using plants remains unexplored in nanotechnology. The plant extract-based synthesis of ZnO nanoparticles has been reported such as *Pelargonium zonale, Punica granatum, Aegle marmelos, Olea ferruginea* and *Berberis vulgaris* (Vahidi et al., 2019; Karaköse et al., 2017; Fowsiya et al., 2019; Hussain et al., 2020; Anzabi, 2018). ZnO nanoparticles have been reported with significant antimicrobial activity, possibly due to the generation of reactive oxygen species (ROS). In the wheat plant, the ZnO nanoparticles induce the formation of free radicles results in an increase in malondialdehyde, reduced lower level of glutathione and reduction in chlorophyll content (Aarti et al., 2006).

Weed is one of the biggest threats to agriculture by reducing the yield of the crop. The irradiations of weeds by traditional methods are time-consuming and the application of chemicals damages plants, causing pollution at an alarming level (Patel, 2011). The weeds are rich in the composition of bioactive components. Hence, this weed can be utilized for preparing fertilizer or herbicide to solve the problems caused by weeds.

Parthenium hysterophorus (Asteraceae) is a destructive, omnipresent herbaceous weed acknowledged for its rapid growth in tropical temperatures. It is native to Southern United States, Mexico, and Central and South America and has now turned into one of the seven most disturbing and precarious weeds globally. In addition to its vigorous growth, it has been reported to cause numerous health threats such as dermatitis, asthma, rhinitis, skin inflammation, hay fever, eczema, allergies, diarrhea to humans and livestock in direct contact. Due to its rampant growth characteristics and systemic toxicity, the management of this weed has become necessary. Appreciably, it can be surveyed for its valuable properties in medicinal applications. This plant is used to treat several diseases, including fever, malaria, neurological disorders, urinary tract infections (Narayanan and Sakthivel, 2010), muscular rheumatism, vermifuge (Sindhura et al., 2014), and so on. It is also used as an anti-parasitic agent (Patel, 2011).

The green synthesis of *P. hysterophorus* upholds this deleterious weed to be cherished for nanotechnology grounded industries in the future. Hence the present investigation was carried out to synthesis ZnO nanoparticles using *P. hysterophorus* plant extract and its various applications as an antimicrobial agent and vegetative growth.

2. Materials and methods

2.1. Collection of samples

Fresh leaves of *Parthenium hysterophorus* were collected from Adhiyaman Arts and Science College. The identification of the plant was carried out with the help of Flora of the Presidency of Madras. The leaves were washed thoroughly, shade dried and ground to a fine powder for further studies.

2.2. Aqueous extract

The aqueous extraction of *P. hysterophorus* was prepared by adapting the procedure published by the method of Datta et al. (2017). 10 g of fine powder was soaked in 100 mL of double-distilled water for 15 min at 60 °C. The aqueous extract was filtered with Whatman No:1 paper before the synthesis of nanoparticles.

2.3. Green synthesis of ZnO nanoparticles

At first, a mixture of 1 mM of zinc nitrate solution and leaf extract in the ratio 9:1 was taken. Then, this mixture was heated at 90 °C with rapid stirring at 800 rpm for 8 h. Finally, we obtained the as-prepared sample of Zn $(OH)_2$. After that, this collected as-prepared sample was annealed at 400 °C for 3 h. Consequently, whitish ZnO nanopowder was gathered, and it was grounded with the help of Cole-Parmer mortar and pestle. Subsequently, this derived sample was stored at the vacuum desiccator chamber and used as characterization and experimental applications.

2.4. Characterization of ZnO nanoparticles

Synthesized ZnO nanoparticle was analyzed by Shimadzu UV–vis spectrophotometer (UV-1800) with the scanning recorded from 190 to 800 nm. The FT-IR spectra were recorded in the range of 4000–400 cm^{-1}. Powder X-ray diffraction (XRD) pattern was obtained using XPERT-PRO PAN analytical diffractometer with Cu $K\alpha$(1.5406 Å) with nickel monochromator and radiation operating at 40 kV and 30 mA. Data were collected in the 2θ range from 10 to 80°, step size 0.05°, and scan step10.16 s. The surface topology with elemental analysis of ZnO nanoparticles was investigated by Scanning electron microscopy (SEM). The accurate particle size and structure were evaluated by transmission electron microscopy (TEM).

2.5. Antimicrobial activity of ZnO nanoparticles

Different concentrations (1, 3, 5 and 10 mg) of synthesized ZnO nanoparticles were evaluated for antimicrobial activity by the disc diffusion method (Gopinath et al., 2017). The activity was tested against Gram-positive bacteria (*Staphylococcus aureus, Streptococcus pneumoniae*), and Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae*), and fungal strain (*Candida albicans*) with Amoxicillin (10 mg) antibacterial disc as a positive control.

2.6. Seed germination and vegetative growth test

At first, 10 g of ZnO nanoparticles were mixed with 500 mL of rice starch solution and sonicated with 30 min. After that obtained the uniform dispersion was slurry form. *Sesamum indicium* (n = 100) seeds were soaked in the slurry and kept overnight. Soaked seeds were seeded on pot culture. Before the seeding process, the pH of the soil was set at 6.8. The plants were raised in 20 pots with five replications and untreated seed raised plants were considered for control. The vegetative growth parameters including seed germination, shoot length, root length, number of leaves, fresh and dry weight of shoot and root were evaluated (Farzad Aslani et al., 2014; Gopinath et al., 2014).

3. Results

Synthesized ZnO nanoparticles were investigated by the UV–vis spectrometer, as shown in Fig. 1. An absorption peak showed at 380 nm. The FT–IR spectrum of *P. hysterophorus* leaf extract and synthesized ZnO nanoparticles are shown in Fig. 2. The leaf extract

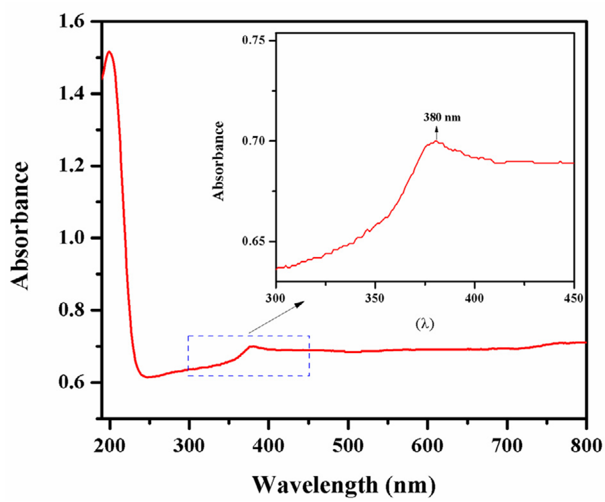

Fig. 1. UV–vis analysis of ZnO nanoparticles.

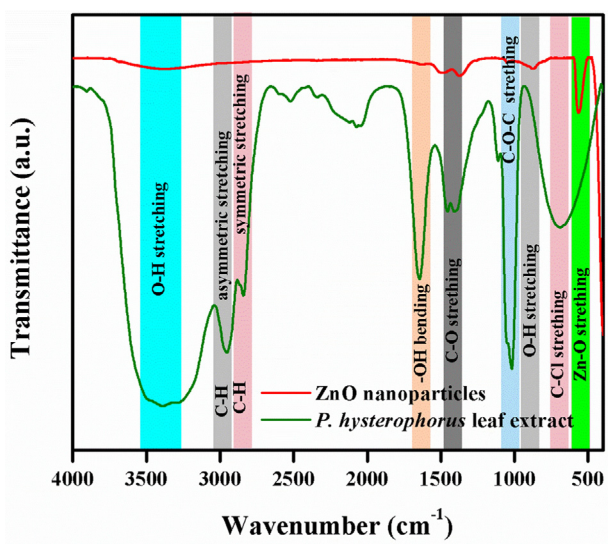

Fig. 2. FT-IR spectral analysis of *P. hysterophorus* leaf extract and ZnO nanoparticles.

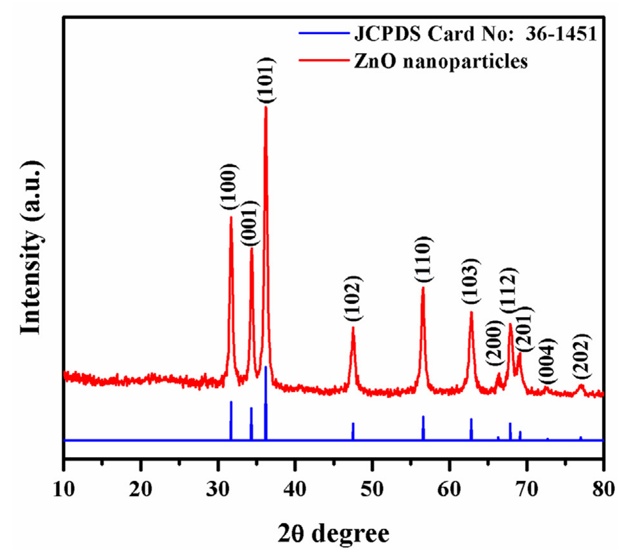

Fig. 3. XRD analysis of ZnO nanoparticles.

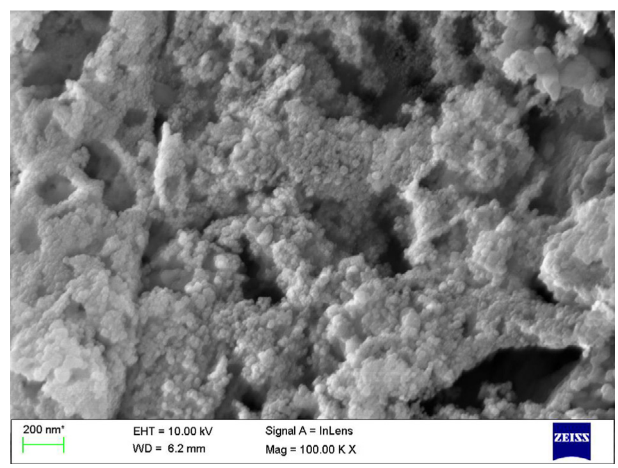

Fig. 4. SEM analysis of ZnO nanoparticles.

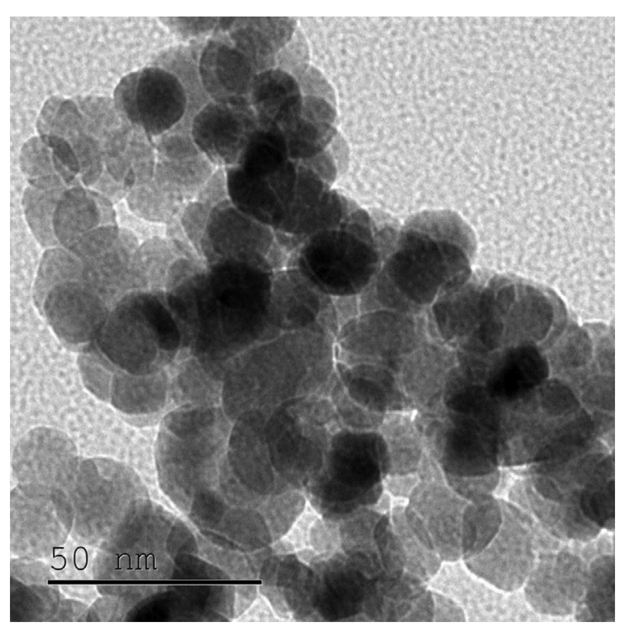

Fig. 5. TEM analysis of ZnO nanoparticles.

spectrum showed vibration bands observed at 3388, 2952, 2838, 1648, 1396, 1015 and 679 cm^{-1}. Depicted Fig. 3, XRD analysis of synthesized ZnO nanoparticles exhibited peaks appeared at 2θ values of 31.68, 34.33, 36.15, 47.45, 56.49, 62.76, 66.29, 67.85, 68.99, 72.54, and 76.89°. SEM and TEM micrographs revealed the spherical shape and 5 ~ 10 nm, as shown in Figs. 4 and 5.

The antimicrobial efficacy of ZnO nanoparticles was shown in Fig. 6. The 10 mg concentration showed a maximum inhibition zone and was observed against *E. coli* (15.00 ± 0.00 mm) followed by *K. pneumoniae* (13.33 ± 0.33 mm). The reduced activity was observed for *S. aureus*, *S. pneumoniae* and *C. albicans* at (12.66 ± 0.16 mm), as shown in Fig. 7. The ZnO nanoparticles treated *S. indicum* seeds were evaluated by the germination of seeds and various vegetative growth (shoot and root length, fresh and dry weight of root and shoot, the number of leaves and plant height) constraints 30 days after sowing (DAS) are summarized in Table 1.

4. Discussion

Synthesized ZnO nanoparticles exhibited an absorption hump at 380 nm due to the absorption of a photon and the excitation

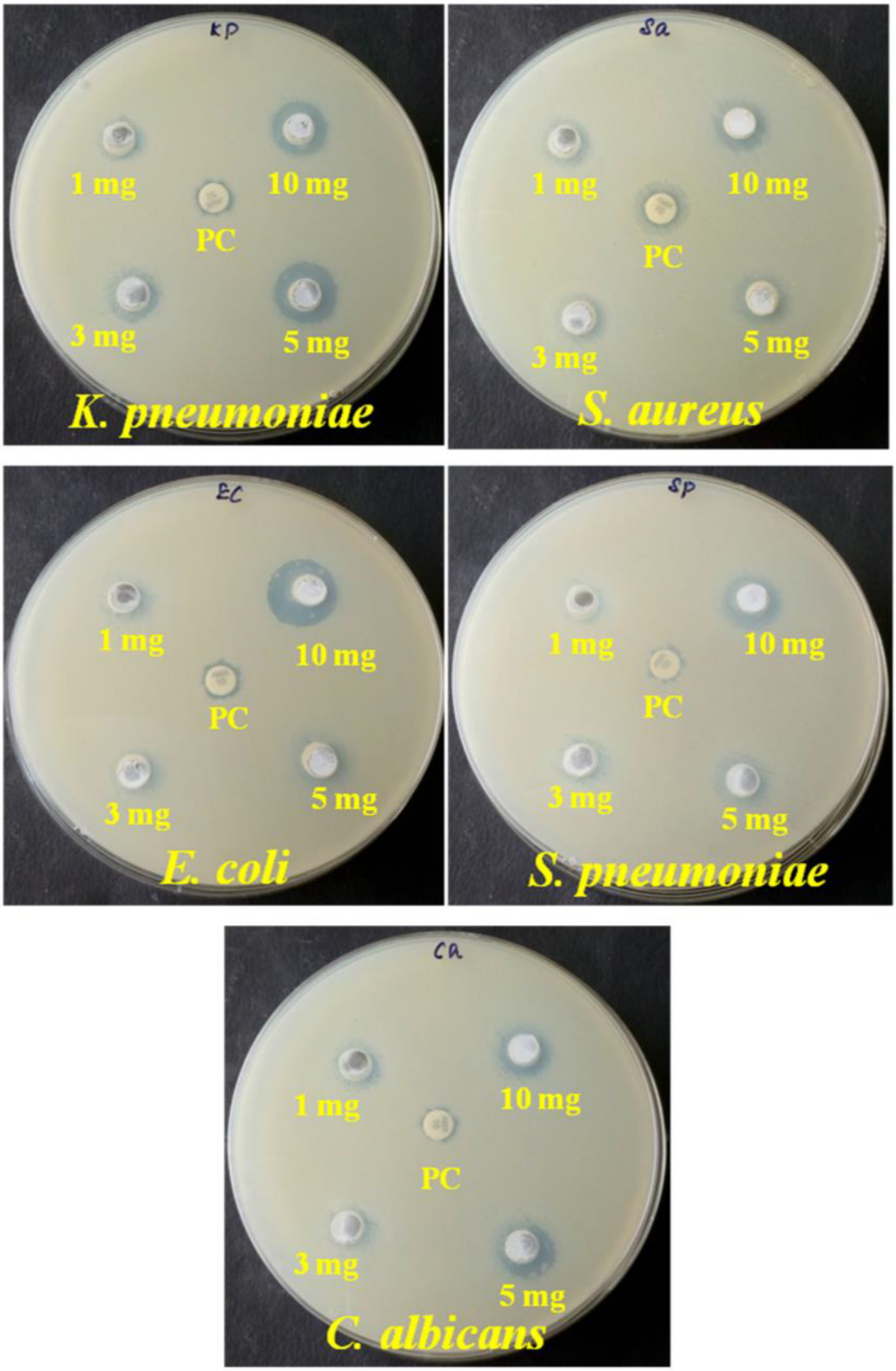

Fig. 6. Antimicrobial activity of ZnO nanoparticles.

of an electron from the valence band to the electron/hole pair generating by the conduction band. Subsequently, the bandgap was estimated by the bandgap Tauc plot calculation and it showed 3.26 eV. Commonly, the optical band gap decreases when the absorption edge shifts towards a longer wavelength. However, the bandgap decrease, whereas relatively increases the better conductivity. Similarly, *Justicia procumbense* and *Rubia cordifolia* mediated ZnO nanoparticles showed an absorption peak at 370 and

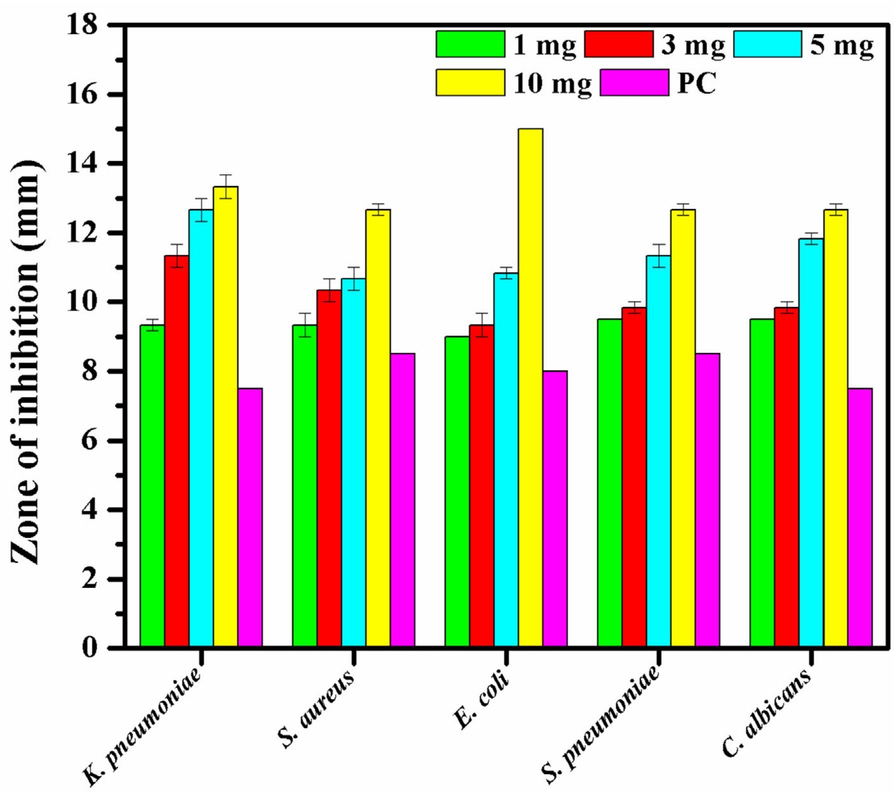

Fig. 7. Different concentrations (1, 3, 5 and 10 mg) of ZnO nanoparticles tested against bacterial and fungal stains, and PC-positive control (10 mg: Amoxicillin).

Table 1
Effect of ZnO nanoparticles on seed germination and vegetative growth parameters of *S. indicum* at 30 days after sowing.

	a (%)	b (cm)	c (cm)	d (n)	Fresh weight		Dry weight	
					Shoot (mg/g)	Root (mg/g)	Shoot (mg/g)	Root (mg/g)
Control	96	09.4 ± 0.01	05.2 ± 0.01	20.10 ± 0.31	09.9 ± 0.01	05.5 ± 0.01	02.4 ± 0.01	01.3 ± 0.01
Treatment	99	18.0 ± 0.01	10.1 ± 0.01	25.6 ± 0.31	15.3 ± 0.01	10.2 ± 0.01	03.8 ± 0.01	02.5 ± 0.01
C.D		0.03	0.03	0.68	0.03	0.03	0.03	0.03
	(<5%)							

C.D. – Critical Difference
a – Seed Germination
b – Shoot length
c – Root length
d – Number of leaves

374 nm (Umavathi et al., 2020; Sisubalan et al., 2018). The present result matched these results. FT–IR spectrum of *P. hysterophorus* leaf extract showed vibration bands observed at 3388, 2952, 2838, 1648, 1396, 1015 and 679 cm^{-1} corresponds to O–H stretching, C–H asymmetric stretching, C–H symmetric stretching, bending vibrations of –OH, –C–O stretching, –C–O–C stretching and C–Cl stretching, respectively. These functional groups are associated with the phytoconstituents of flavonoids, phenolics, tannin and phytic acid (Parihar et al., 2015). The ZnO nanoparticles spectrum revealed vibration bands observed at 3410, 870, and 573 cm^{-1} due to the O–H stretching, C–H bending, and weak Zn–O stretching, respectively. At the same time, 573 cm^{-1} vibration band ascribed to the wurtzite structure of ZnO. A similar trend was observed in the previous report (Kasi and Seo, 2019). XRD analysis provides information about crystallinity and phase structure. As shown in Fig. 3, XRD analysis of synthesized ZnO nanoparticles exhibited three high-intensity peaks that appeared at 2θ values of 31.68, 34.33, 36.15 is owing to the (100), (001), and (101) planes which indicated the hexagonal wurtzite structure of ZnO, respectively, according to the JCPDS card # 36-1451. Subsequently, small-intensity peaks are seen at 2θ values of 47.45, 56.49, 62.76, 66.29, 67.85, 68.99, 72.54, and 76.89° attributed to (102), (110), (103), (200), (112), (201), (004) and (202) planes, respectively. Remarkably, there is an additional peak was not detected, which indicated the green synthesis process the purity ZnO nanoparticles. The crystallite size of ZnO nanoparticles was calculated by Scherrer's equation $D = 0.94\lambda/\beta\cos\theta$ (Kasi et al., 2019), and it showed the mean value of crystallite size at 17.63 nm. The surface topology and particle distribution were investigated by the SEM micrographs, as shown in Fig. 4. SEM micrograph indicates a homogeneous distribution with a spherical shape. The accurate size and shape of nanoparticles were studied

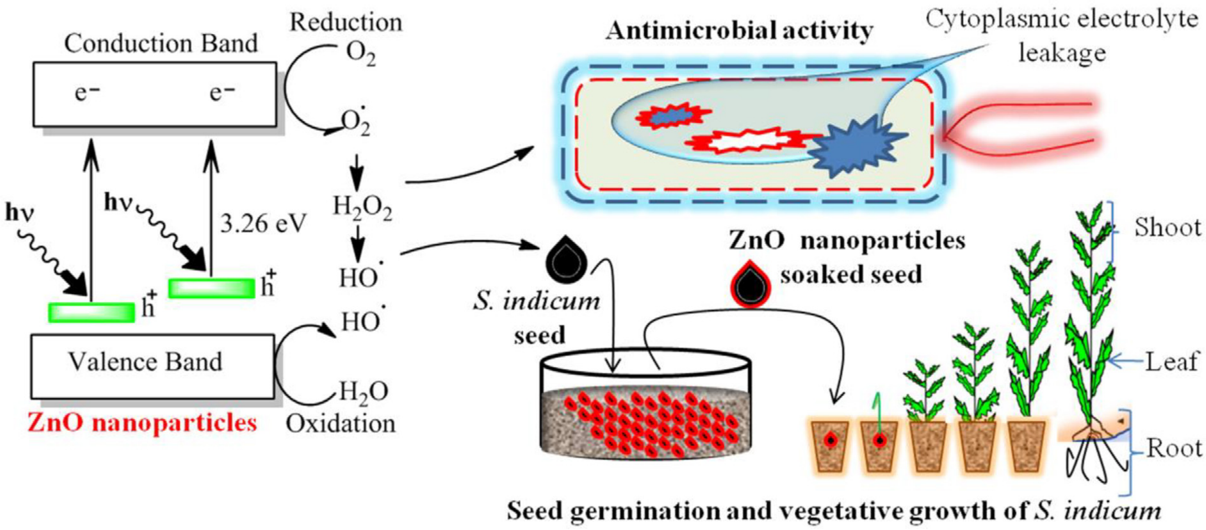

Fig. 8. Schematic representation of antimicrobial and ZnO nanoparticles impact on seed germination and vegetative growth of *S. indicum*.

by TEM analysis. TEM micrograph exhibits the spherical shape with the range between 5 and 20 nm and it showed an average size of 10 nm (Fig. 5).

4.1. Antimicrobial activity

The ZnO nanoparticles were effectively inhibited at both bacterial and fungal growth are shown in Fig. 6. The 10 mg concentration showed a maximum inhibition zone. However, in our antimicrobial activity results indicated an increase with increased ZnO nanoparticles concentration, as well as the zone of inhibition depended on the species specificity and particle size of ZnO nanoparticles (10 nm) was a major impact on antimicrobial activity, which is due to the high surface and volume-relation. Besides, antimicrobial activity is related to the bacterial and fungal cell wall structure, ribosome sub-unit, and intercellular original: (mesosome for bacteria and mitochondria for fungus). The positively charged ZnO nanoparticles and negatively charged bacterial and fungal walls can make binding by the electrostatic interaction.

Consequently, cell wall-bounded ZnO nanoparticles cause pith formation of the microbial cell wall and loss the cell membrane integrity (Umavathi et al., 2020; Kim et al., 2020; Viswanathan et al., 2020). Whereas Zn^{2+} ions penetrated to the cytosol and binding with sulfur-containing amino acids, interference the bio-signaling affected the DNA replication, inactivated the electron transport chain, and reduced the ATP synthesis, causes mesosoma oxidative stress and mitochondrial oxidative stress. Overall, imbalanced metabolic activities and leaked out the biological electrolyte lead to microbial cell death (Fig. 8) (Kasi and Seo, 2019; Kasi et al., 2019).

4.2. ZnO nanoparticles impact on seed germination and vegetative growth of S. indicum

The ZnO nanoparticles treated *S. indicum* seed germination and growth parameters such as shoot and root length, fresh and dry weight of root and shoot, the number of leaves and plant height exhibit remarkable improvement as compared to control (Table 1). More importantly, shoot length increased from 09.4 to 18.0, root length 5.2 to 10.1 and the number of leaves increased from 20.1 to 25.6 compared to control. The dry and fresh weight of both root and shoots also reported significant improvement. In our finding concluded that ZnO nanoparticles migrate with *S. indicum* seeds coat by the soaking. Through the imbibitions process, water with ZnO nanoparticles penetrated to the cytosol and involved the metabolism by shifting the catalase, superoxide dismutase, peroxidase, phenol, similar (ROS), protein and chlorophyll content formation (Ruttkay-Nedecky et al., 2017). On the other hand, it can act as a micronutrient. Because ZnO nanoparticles contact with *S. indicum* root hair, it continuously releases the Zn^{2+} ions on ZnO nanoparticles surface. Zn^{2+} ions were uptake by the active transport to the plant cell. Enzymes encompassing Zn^{2+} ions are crucial for electron transport, ATP generation, Chlorophyll bio-synthesis, and maintaining membrane integrity (DalCorso et al., 2014). Zn^{2+} ions are activated the TATA BOX and promote the DNA replication towards the protein synthesis, which is due to the Zn^{2+} ions act as metal regulating protein. The intercellular plant origin of peroxisomes involved in breaking down the toxic molecule of H_2O_2 was quickly converted into oxygen and water. The oxygen mainly accumulates at the meristematic region and is needed for cell division, while H_2O_2 confers the differentiation and accumulates in the elongation zone (Barrena et al., 2009). The reasons mentioned above have been induced by the vegetative growth of *S. indicum* (Fig. 8).

5. Conclusion

Overall, green synthesis of ZnO nanoparticles using *P. hysterophorus* leaf extract by the single-step process. The optical property of synthesized ZnO nanoparticles exhibited UV–vis absorption at 380 nm with a bandgap value of 3.26 eV whereas, FT-IR spectrum revealed at 573 cm^{-1} vibration band correspond to the weak Zn-O stretching. XRD result confirms the formation of the hexagonal wurtzite structure of ZnO. SEM and TEM micrographs were revealed homogeneous distribution with spherical shape and an average size of 10 nm. The antimicrobial activity result indicates a concentration-dependent effect on both bacterial and fungal strains. However, 10 mg concentration exhibit excellent antimicrobial activity. ZnO nanoparticles treated *S. indicum* seeds significantly promote seed germination and vegetative growth. Zn^{2+} ions induce the enzyme activities and the toxic by-product of H_2O_2 was converted into oxygen and water. Appreciably, the particle size, shape, crystallite size are major roles for all activities. However, *P. hysterophorus* weed plant can be used for another metal oxide nanoparticles synthesis, whereas aggressive dominance of species in an environment leads to minimizing and considering an alternative approach for physical and chemical synthesis of nanoparticles.

Declaration of Competing Interest

None.

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