



# One-pot green synthesis of ZnO nanoparticles using *Scoparia Dulcis* plant extract for antimicrobial and antioxidant activities

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## Abstract

Nanostructured Zinc oxide (ZnO) materials have attained exciting research interests among various metal oxide nanoparticles due to their unique features. Thus, the scope of applications for ZnO nanoparticles (ZnO NPs) is vast and efficient. The current study demonstrates a simple and environmental-friendly approach for the synthesis of ZnO NPs using the extract of the *Scoparia Dulcis*. *Scoparia Dulcis* is a common medicinal plant in Kerala (India) that is traditionally used for its medicinal properties. Morphological characterizations of the as-synthesized ZnO NPs were evaluated using X-ray diffraction, Fourier transform infrared spectroscopy (FTIR), and field-emission scanning electron microscopy (FESEM). The results revealed that ZnO NPs showed pebble-like morphology and possessed an average particle size of ~20 nm. Further, antibacterial and antifungal activities of as-prepared ZnO NPs were investigated against *E. coli*, *Staphylococcus aureus*, as well as *Candida albicans*, and *Aspergillus niger*, respectively, using the agar-well diffusion method. The results revealed that the prepared ZnO NPs shows excellent antimicrobial activity against the examined microorganisms. Moreover, the antioxidant activity of the as-synthesized ZnO NPs was evaluated using the DPPH assay, which indicated an excellent IC<sub>50</sub> value of 1.78 µg/mL that shows high antioxidant activity. All these results proved that the *S. dulcis* plant extract-mediated synthesis method is a simple, low-cost, eco-friendly procedure for preparing efficient ZnO NPs for biomedical applications.

**Keywords** Zinc oxide nanoparticles · Green synthesis · *Scoparia Dulcis* · Antimicrobial activity · Antioxidant activity

## Introduction

Nanomaterials with diverse structures and properties have attracted exciting research attention over the past decades. The Discovery of novel nanomaterials which can be used for multifunctional applications are the thrust area of research in nanotechnology (Zhang 2015). Nanomaterials that can be used as antimicrobial agents have significant importance owing to their action against health-threatening pathogens (Zheng et al. 2018). Antimicrobial activity has been identified in several nanomaterials that can be exploited to build healthcare goods for biomedical applications such as wound dressing materials, biosensors, drug carriers, etc. (Umar et al. 2007; Mihai et al. 2019; Gupta and Roy 2020).

Due to their great performance in various applications, ZnO NPs can be considered as a centre of focus among metal oxide nanoparticles in different applications like sensors, cancer theranostics, photodegradation, optoelectronics, and recently an antiviral agent against COVID-19 (Solanki et al. 2009; Muchuweni et al. 2017; Hamdi et al. 2021).

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One unique feature that makes ZnO NPs is their biocompatibility which makes them ideal candidates to employ in real-time applications of personal healthcare products such as anti-dandruff shampoos, toothpastes, sunscreens, and other modern cosmetics (Sogne et al. 2017). All these features make ZnO NPs, the third position of global production among various metal nanoparticles (Piccinno et al. 2012). However, it has been noted that the properties of ZnO NPs were influenced by unique morphological and structural features which can reflect in their performance, like biological action and electrical properties (Kołodziejczak-Radzimska and Jesionowski 2014). A specific morphology for the ZnO NPs depends on various parameters employed in the synthesis procedure. Conventional synthesis methods for preparing ZnO NPs can be divided into two broad approaches such as (i) bottom-up and (ii) top-down strategies. These methods usually employ precursor materials and proceed through a series of physical and chemical processes that lead to the final desired nanomaterial. However, both these conventional methods suffer from their own limitations, such as the involvement of high energy processes, large amounts of chemical reagents, lack of good yield, and high cost for the synthesis process. All these limitations of classical methods paved the researchers to explore novel preparation methods with less cost and energy demands like one-pot methods.

The challenges and drawbacks faced in the conventional synthesis methods can be successfully resolved by the application of 'green' approaches, which involve the application of biomaterials like plant extracts, microorganisms, and biowaste materials (Jain et al. 2020; Pillai et al. 2020; Basumatari et al. 2021). Microwave and ultrasound-assisted synthesis approaches were also reported successfully, but these methods also suffer from energy demand (Bayrami et al. 2019). Due to these reasons, green synthesis methods that use plant extract as the preparation agent for ZnO NPs from precursors are getting significant attention. There are various advantages to adopting plant extract-assisted synthesis methods for preparing ZnO NPs. These include a one-pot synthesis strategy, lack of other chemical reagents and solvents, very low cost, etc., (Akintelu and Folorunso 2020). In addition, there are significant advantages for the plant extract-mediated synthesis method over conventional wet-chemical synthesis approaches for synthesizing ZnO NPs. The phytochemicals present in the plant extract could serve as in situ reducing and stabilizing agents during the synthesis process. Further, the method uses only an aqueous medium and does not employ any toxic chemicals or solvents. Thus, the present study brought an eco-friendly approach. This method can be successfully implemented for synthesizing ZnO NPs for biomedical applications due to the lack of toxic chemicals (Dulta et al. 2022). It has also been reported that plant extract-mediated synthesis produces

more stable NPs compared to that synthesized using organic solvents (Gomathi and Suhana 2021).

For these reasons, plant extract-assisted synthesis methods to synthesize nanomaterials are among the effective choices among green synthesis methods to implement a sustainable and cost-effective preparation strategy for preparing ZnO NPs. For this, plant extracts from various medicinal plants are effective such as Aloe vera, Ocimum, Neem, Pepper, etc., (Ali et al. 2016; Abinaya et al. 2020; Nayak et al. 2020). Furthermore, the formation of metal oxide NPs from the precursor will be facilitated by the presence of more quantity of phytochemicals such as flavonoids and terpenes. The ZnO NPs prepared using these methods were found to possess good action against various common pathogens encountered in the healthcare field (Happy Agarwal et al. 2018). Also, they showed synergistic activity when used with antibiotics with an appreciable extent of antimicrobial potential (Patra et al. 2014).

Herein, we have designed a simple, low-cost, eco-friendly, and one-pot green synthesis strategy for preparing ZnO NPs by employing leaf extract of *Scoparia Dulcis* medicinal plant. The plant *S. dulcis* in *plantaginaceae* family is well known for its diverse therapeutic properties. It includes anti-diabetic, antioxidant, anti-analgesic, and anti-inflammatory activity (Jiang et al. 2021). Also, *S. dulcis* is traditionally used as a medicine for urolithiasis owing to its excellent anti-urolithiasis activity. In-vitro studies revealed the inhibitory mechanism of yeast  $\alpha$ -glucosidase by the aqueous extract of *S. dulcis*. Recently, the protective ability of *S. dulcis* against metabolic disorders has been elucidated and analyzed in detail. All these diverse medicinal activities are attributed to the presence of an enormous number of phytochemicals in *S. dulcis*, which forms the molecular basis of activity against various diseases. Phytochemical screening of the plant revealed that the major class of chemical constituents include steroids, flavonoids, nitrogen-containing compounds, terpenoids, and phenolics.

Interestingly, clinical studies are also in rapid progress to apply the medicinally active composition of *S. dulcis* to create pharmaceutical products, especially anti-diabetic drugs. Silver nanoparticles were synthesized utilizing *S. dulcis* extract in recent investigations. It has been observed that the synthesized nanoparticles were active against various microbes. However, no reports of *S. dulcis*-derived ZnO NPs have been found (Nguyen et al. 2020).

This is the first report on using *S. dulcis* extract to synthesize ZnO nanostructured material. The synthesized ZnO NPs have antibacterial and antifungal capacity, which were analyzed on *Staphylococcus aureus* and *E. coli* as two prominent bacterial species and antifungal activity with two common fungi (*Candida albicans* and *Aspergillus niger*). Further, the antioxidant activity was also investigated through DPPH

assay. Overall, we report a facile green synthesis strategy to fabricate ZnO NPs for multifunctional biomedical purposes.

## Materials and methods

### Materials

*Scoparia Dulcis* plants were obtained from the western ghats of Kerala, India. All the chemicals used in the study, such as Zinc nitrate hexahydrate ( $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Ascorbic acid, Methanol, and KOH were purchased from Merck, India. All the experiments were conducted in double-deionized water.

### Plant extract preparation

2 g of fresh *S. dulcis* leaves were collected and rinsed multiple times with distilled water to remove impurities. It is then cut into small pieces, followed by ultrasound irradiation for 10 min in 50 mL water to migrate active compounds to the medium. The plant extract was obtained after filtering through Whatman filter paper, followed by centrifuging the resultant solution. This extract was collected and used to prepare ZnO NPs.

### Fabrication of ZnO NPs

Plant extract (25 mL) was added to the beaker while heating at 60 °C; under constant stirring, 2 g of  $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  was added to the extract. The mixture is then kept for stirring and boiling until the water is reduced to form a paste with a light brown shade. This paste is collected, then calcined

at 450 °C for about two hours to get ZnO NPs witnessed by the formation of white-colored powder. The final yield of the material is 0.45 g. The overall scheme for the synthesis is represented in Fig. 1.

### Characterization of ZnO NPs

$\text{CuK}\alpha$  radiation ( $\lambda = 1.54 \text{ \AA}$ ) using Bruker D8 Advance Powder XRD diffractometer was employed for analyzing XRD data. FTIR spectra were recorded using SHIMADZU DR 43 S spectrophotometer. The material's morphology was determined using FESEM (Carl Zeiss, Sigma).

### Estimation of antibacterial and antifungal activity

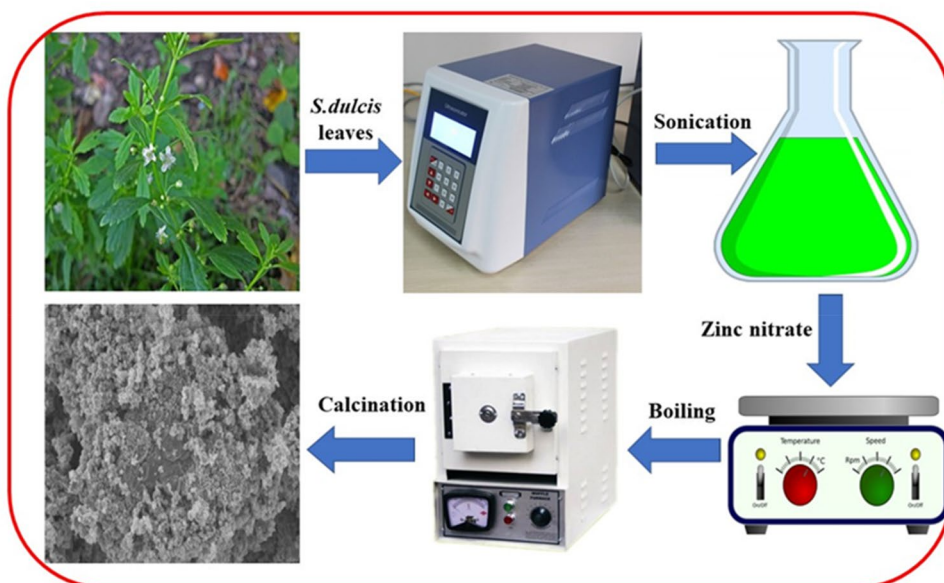
#### Collection of microorganisms

*Staphylococcus aureus-902* (Gram-positive bacteria), *E. coli-443* (Gram-negative bacteria), *Candida albicans*, and *Aspergillus niger* were purchased from MTCC, Chandigarh, India. Positive controls for antibacterial and antifungal assays were Nutrient Agar medium, Nutrient broth, and Gentamicin and Amphotericin B antibiotic solutions supplied from Himedia in India. All analysis was done using the agar-well diffusion method and according to the standard protocols (Bauer et al. 1959a, b).

#### Antibacterial assay

The nutrient medium was made by dissolving 2.8 g of commercial Nutrient Agar in 100 mL double distilled water. This is ramped for 15 min in an autoclave for 121 °C at 151bs pressure. The medium is then mixed well and transferred

**Fig. 1** Scheme of ZnO NPs preparation using *S. dulcis* leaf extract



into 100 mm petri-dishes. The bacterial strains (*S. aureus* and *E. coli*) were seeded into these petri plates for 24 h. Different concentrations of ZnO NPs were added in order of 500, 250, 100, and 50  $\mu\text{g/mL}$  to the wells cut from the bacterial culture, followed by 24-h incubation at 37 °C. Graph Pad Prism 6.0 software was employed to measure the zone of inhibition (ZOI) values.

### Antifungal assay

1.5 g of Agar, 20 g of potato infusion, 2 g of dextrose were well mixed and dissolved in 100 mL double distilled water to prepare the potato dextrose agar medium. The dissolved medium was ramped for 15 min at 121 °C and 15 lbs pressure. The medium was then transferred into 100 mm petri-dishes. These Petri plates were planted for fungal strain (*Candida albicans* and *Aspergillus niger*), and the wells were cut & ZnO NPs with varying concentrations such as 500, 250, 100, and 50  $\mu\text{g/mL}$ , finally incubated at 28 °C for 72 h. Zone of inhibition were calculated similarly to the procedure done for bacterial culture.

### Antioxidant activity

In methanol, 0.1 mM DPPH solution was produced, and 100  $\mu\text{l}$  of this solution was mixed to 300  $\mu\text{l}$  of ZnO NPs dispersion at various concentrations, including 500, 250, 100, 50, and 10 g/mL. Vigorous shaking was done to get a uniform dispersion and kept aside for 30 min at room temperature. Absorbance values at 517 nm were measured using a UV–VIS spectrometer using Ascorbic acid as the standard. Triplicate analysis was done for all the samples to confirm concordant results.

## Results and discussion

### XRD

The XRD data can establish the crystallinity and purity of the nanostructures. XRD patterns are the results of the diffraction process of the X-rays on various crystallographic planes of the system. Figure 2 depicts the XRD pattern of ZnO NPs. The synthesized ZnO NPs have good crystallinity and purity, which are evident from the sharp peaks.

The diffraction peaks were found to arise from the miller planes of (100), (002), (101), (102), (110), (103), (112), which corresponds to the angle  $2\theta = 31.7^\circ$ ,  $34.7^\circ$ ,  $36.2^\circ$ ,  $47.4^\circ$ ,  $56.7^\circ$ ,  $63.1^\circ$ ,  $68.1^\circ$  respectively. This confirms that the ZnO NPs possess a hexagonal phase consistent with the Wurtzite structure [JCPDS No: 89-7102] (El-Belely et al. 2021). The XRD data indicate that plant extracts significantly impacted the development of ZnO NPs with good

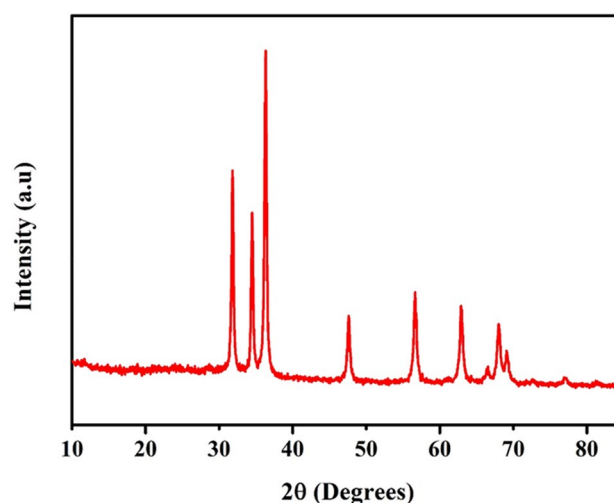


Fig. 2 XRD data of ZnO NPs

crystalline nature, as evident from the peak intensities (Pillai et al. 2020). Further investigations to the structure using the Scherrer equation divulge that ZnO NPs have an average particle size  $\sim 20.2$  nm. The surface crystallization of organic constituents from the plant extract on ZnO NPs is responsible for the presence of minute peaks (El-Belely et al. 2021).

### FTIR analysis

Phytochemicals present in the bioresource employed for the synthesis are reported to be responsible for the formation of nanoparticles from their metal precursor. Different classes of phytochemicals such as flavonoids, chalcones, anthocyanins can aid in the formation of ZnO NPs from precursors (Jayachandran et al. 2021). The evidence of these moieties can be easily obtained from the FTIR spectra of the sample, along with information about the vibrational modes of the ZnO NPs as represented in Fig. 3. The presence of ZnO from inter-atomic vibrations of the lattice is confirmed by the peak at  $835\text{ cm}^{-1}$ . Phytochemicals present in the plant extract give the rest of the peaks. All these peaks are in accordance with green synthesized ZnO NPs reported in the literature. The vibrational band above  $3000\text{ cm}^{-1}$  arises from the O–H bond stretching and can be due to the phenolic contents present in the extract of *S. dulcis*. The vibration at  $2340\text{ cm}^{-1}$  occurs from the nitrile group (CN) expected from the nitrogen compounds of the plant. The carbonyl (C=O) bond of unsaturated carbonyl compounds and stretching of C=C bonds from aromatic moieties are linked to the peak at  $1680\text{ cm}^{-1}$ . Stretching of C–H mode for alkanes are responsible for  $1350\text{ cm}^{-1}$ . These results implies the role of phenolic, flavonoids, and other phytochemical compounds present in the *S.aureus* extract can serve as the bio-reductant through stabilization of the zinc salts which finally gives control of

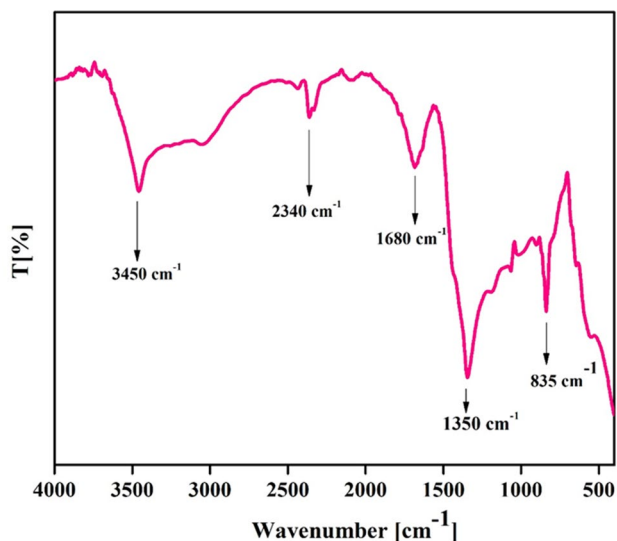


Fig. 3 FTIR spectrum of ZnO NPs

the ZnO size on green synthesis (Elumalai and Velmurugan 2015). Thus, it is clear that ZnO NPs are produced from the metal precursor through the phytochemical constituents present in plant extract as a reducing agent.

It has been reported that several phytochemicals present in the plant extract serve as stabilizing and reducing agents for forming ZnO NP's from the precursor. The hydroxy and oxo substituents on the plant metabolites can coordinate with the  $Zn^{2+}$  ion, making the process feasible. Here we demonstrate a plausible mechanism synthesis of ZnO NPs using precursors through the phytochemicals from *S. dulcis* as depicted in Fig. 4. 2-Hydroxy-2H-1,4-benzoxazol-3-one was selected as the phytochemical for proposing the mechanism since this is an active constituent present in all parts of the *S. dulcis* (Jiang et al. 2021). The mechanism involves deprotonation of the hydroxyl functional group, which creates a negatively charged oxygen. The zinc ion is getting chelated to this charge center through the formation of the complex, which finally produces ZnO NPs inconsistent with previously reported mechanisms in literature (Thi et al. 2020; Ansari et al. 2020; Selim et al. 2020).

### FESEM analysis of ZnO NPs

The structural characteristics and surface morphology of ZnO were studied using field-emission scanning electron microscopy. Figure 5 shows the FESEM pictures of the green-produced ZnO NPs.

The low-resolution image (Fig. 5a) indicates significant agglomeration for the ZnO NPs which is a characteristic feature of the plant extract-mediated synthesis. On the other hand, the high resolution in the image (Fig. 5b) clearly represents that ZnO NPs possess pebble-like morphology at

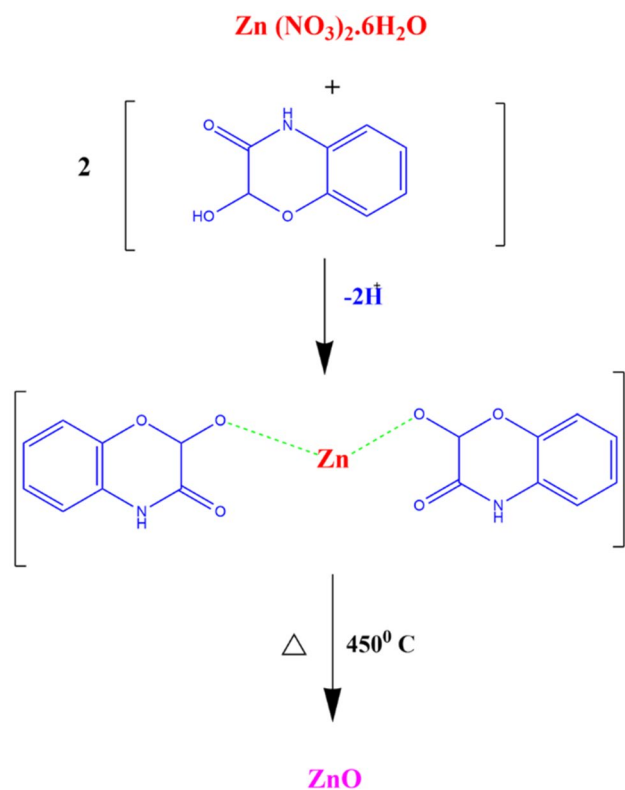


Fig. 4 *S. dulcis* extract-induced production of ZnO NPs: a possible mechanism

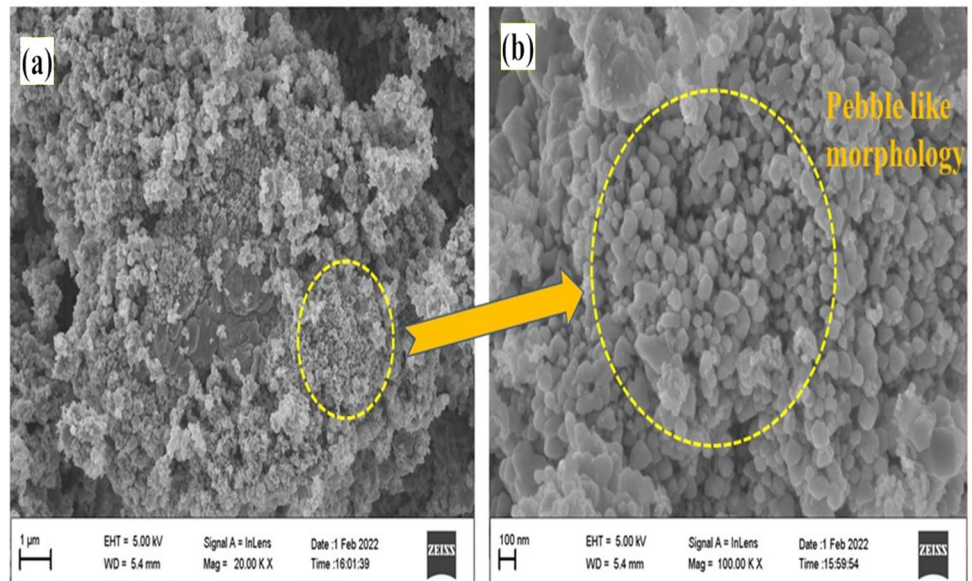
the nanoscale. These nanopebbles are not homogenous and thus possess a diverse size range. The reason for agglomeration can be due to the existence of phytochemical moieties on the surface of the particles or due to the experimental conditions in the synthesis like pH of the medium, temperature etc., (Bandeira et al. 2020). The change in temperature affects crystal growth by variation in the nucleation process. Also, the increase in pH of the reaction medium from acidic to neutral and to the basic range will cause a decrease in agglomeration (Alias et al. 2010). This can be correlated to the slightly acidic nature of the *S. dulcis* leaf extract, which may cause slight agglomeration as observed in the ZnO NPs morphology.

### ZnO Nps activity against bacteria & fungi

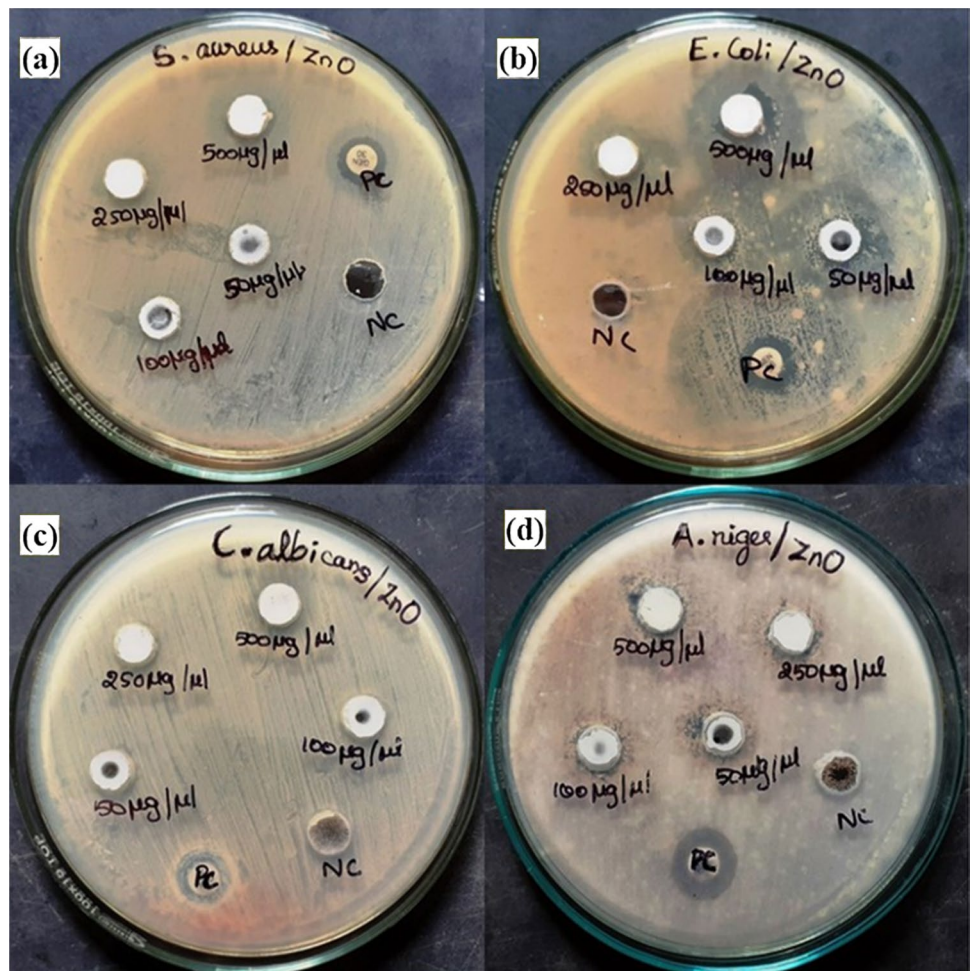
Agar well diffusion method was employed for antibacterial and antifungal activity. In this method, diffusion of ZnO NPs into the medium was allowed to make interaction with the test organisms seeded in the petri-plates (Bauer et al. 1959a, b). As a result of the confluent lawn of development, the zones of inhibition (ZOI) will be uniformly round, as shown in Fig. 6.

ZOI is measured in millimeters which gives the extent of antimicrobial action of the sample against

**Fig. 5** ZnO NPs with pebble-like shape as shown by FESEM



**Fig. 6** Inhibition activity of ZnO NPs on **a** *S. aureus*, **b** *E. coli*, **c** *C. albicans* and **d** *A. niger*



respective pathogens. The antibacterial activity was analyzed using *Staphylococcus aureus* and *Escherichia coli* for

gram-positive and gram-negative stains, respectively. Also, the antifungal activity was analyzed using two common

fungal pathogens such as *Aspergillus niger* and *Candida albicans*. Table 1 shows the ZOI values in different concentrations (500 µg/mL, 250 µg/mL, 100 µg/mL and 50 µg/mL). PC is positive control used which is a Gentamicin antibiotic.

It is clear that at 500 µg/mL, the ZnO NPs showed slightly higher antibacterial activity than Gentamicin on *S. aureus*. The activity decreases with a decrease in concentration, and a similar pattern is observed for *E. coli* also, which implies the dose-dependent action of ZnO NPs. However, in the case of *E. coli*, the activity was more than *S. aureus* for all the concentrations except 500 µg/mL. Similarly, for the antifungal activity, ZnO NPs showed ZOI values higher than that of Amphotericin B, which was used as a positive control at 500 µg/mL for *C. albicans*. No activity was observed for all the other concentrations, as summarized in Table 2. The results are significantly good on comparing with previous reports (Abdelhakim et al. 2020; Raj et al. 2021).

The activity of ZnO NPs against tested bacteria and fungi were represented in Fig. 7. However, ZnO NPs have shown high activity for *A. niger* for all the concentrations and comparable activity at 50 µg/mL than that of Amphotericin B. This discrepancy in the activities may be because of differences in cell wall compositions and the membrane structure.

Various mechanisms have been accounted for in the literature to explain the action of ZnO NPs against pathogens. The production of significant amounts of reactive oxygen species is one main inhibitory route (ROS). High oxidizing property and reactivity makes ROS to intervene in biological activities. Another study indicates the existence of multiple metabolic pathways for the action of ZnO NPs against microorganisms. This involves various media-dependent biomolecular pathways such as (i) destruction of cell integrity, (ii) ROS species generation, (iii) Zn(II) ion release to the biological species (Espitia et al. 2012). Further, other factors like concentration, morphology, specific surface area and particle size, could also influence the antibacterial action of ZnO NPs (Sirelkhatim et al. 2015). The ROS

species include  $\text{OH}^-$ ,  $\text{H}_2\text{O}_2$ , and  $\text{O}_2^{2-}$  produced on the ZnO surface, which are responsible for the cell death of microbial species through the destruction of cellular components. Here,  $\text{H}_2\text{O}_2$  molecules are mainly responsible for bacterial cell death due to their ability to penetrate to the cell wall and triggering cellular damage.  $\text{OH}^-$  and  $\text{O}_2^{2-}$  cannot penetrate the cell wall due to the electrostatic barrier due to the negatively charged bacterial cell wall, which is not a challenge for  $\text{H}_2\text{O}_2$  permeation. The higher bactericidal activity also results from the continuous release of peroxides by the ZnO NPs in the growth media (Sirelkhatim et al. 2015).

Reports suggest that the action of ZnO NPs against *S. aureus* and *E. coli* is inversely related to the size of ZnO NPs. Therefore, a decrease in the size will lead to better antibacterial activity against both *S. aureus* and *E. coli* which implies the activity is size-dependent. Also, the increased activity with the concentration of ZnO is attributed to the lactate dehydrogenase leakage resulting from mitochondrial dysfunction (Jeng and Swanson 2006).

In our knowledge, detailed mechanistic insights into the action of ZnO NPs against fungus is still in the infant stage, which needs to be elucidated in detail (Sun et al. 2018). Factors like crystallographic parameters and surface charge density also need to be thoroughly investigated to gain insights into biocidal activity. It has been reported that the presence of phytochemical components on the surface of ZnO NPs plays a vital role in enhancing antimicrobial efficiency (Ayoughi et al. 2011). The antifungal action of ZnO NPs could also be due to the disruptive action and permeation of the cell membrane which affects the cell membrane integrity and thereby leading to damage and cell death (Jamdagni et al. 2018).

Our results indicate that the combinations of antibiotics with ZnO NPs would tremendously enhance the activity through synergistic action. The ZnO NPs possess antibacterial and antifungal capacity for both gram-positive and negative stains and against common fungi. However, the

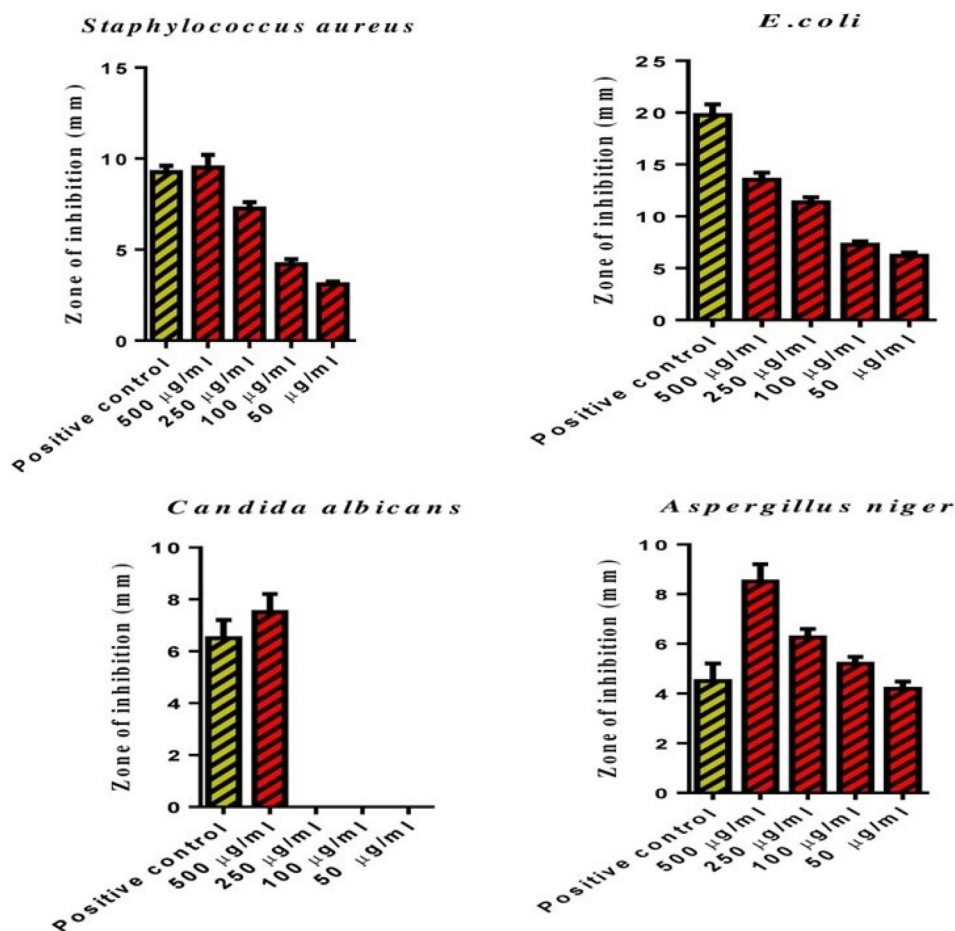
**Table 1** ZOI values of ZnO NPs on *S. aureus* and *E. coli*

S. no.	Name	ZOI (mm) SD ± Mean				
		500 µg/mL	250 µg/mL	100 µg/mL	50 µg/mL	PC
1	<i>Staphylococcus aureus</i>	9.5 ± 0.7	7.25 ± 0.35	4.2 ± 0.28	3.1 ± 0.14	9.25 ± 0.35
2	<i>Escherichia coli</i>	13.5 ± 0.7	11.35 ± 0.49	7.25 ± 0.35	6.2 ± 0.28	19.75 ± 1.06

**Table 2** ZOI of ZnO NPs on *C. albicans* and *A. niger*

S. no.	Name	ZOI (mm) SD ± Mean				
		500 µg/mL	250 µg/mL	100 µg/mL	50 µg/mL	PC
1	<i>Candida albicans</i>	7.5 ± 0.7	0	0	0	6.5 ± 0.7
2	<i>Aspergillus niger</i>	8.5 ± 0.7	6.25 ± 0.35	5.2 ± 0.28	4.2 ± 0.28	4.5 ± 0.7

**Fig. 7** ZOI values of ZnO NPs on **a** *S. aureus*, **b** *E. coli*, **c** *C. albicans* and **d** *A. niger*



cytocompatibility tests of the synthesized nanoparticles have to be studied with normal cell lines to apply for biomedical applications. The results are very useful for applying the ZnO NPs for agricultural sector since some of these pathogens exist as a potential challenge (Julian et al. 2018; Park and Ronholm 2021). These aspects will be covered in our future works.

### Antioxidant activity

The enhanced antioxidant capacity of ZnO NPs can be attributed to the presence of various classes of phytochemicals on the surface derived from the plant extract used for synthesis (Ravichandran et al. 2016). DPPH assay provides a convenient and direct method to determine antioxidant efficacy. Here we have analyzed the in-vitro antioxidant capacity of the prepared ZnO NPs using DPPH free radical

scavenging method. DPPH assay is a very reliable and facile method to monitor the antioxidant potential of nanoparticles (Khorrami et al. 2019). The principle of DPPH assay is that antioxidant activity is directly correlated to radical scavenging action which is reflected as the disappearance of DPPH in the sample under consideration. The disappearance of DPPH is monitored through a UV–visible spectrometer which directly gives the extent of antioxidant activity. A strong absorption maximum at 517 nm with purple color is attributed to DPPH (Table 3). The synthesis of DPPH is confirmed by yellow color formation when hydrogen is absorbed from an antioxidant that is stoichiometric in terms of the quantity of hydrogen atoms consumed in the process. Higher radical scavenging ability is reflected through lower absorbance values of the reaction mixture. As a result, the antioxidant activity can be determined using the following formula while monitoring the decrease in UV absorption at 517 nm.

$$\text{DPPH scavenging effect (\% inhibition)} = \frac{\text{Absorbance of control} - \text{Absorbance of reaction}}{\text{Absorbance of control}} \times 100.$$



**Table 3** DPPH assay values (percentage of inhibition) of ZnO NPs

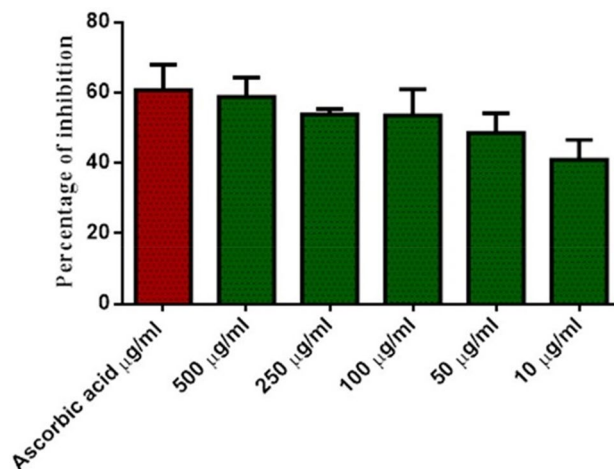
S. No	Tested sample concentration (µg/mL)	Percentage of inhibition (%)
1	Ascorbic acid	64.85
2	500 µg/mL	56.60
3	250 µg/mL	54.02
4	100 µg/mL	49.18
5	50 µg/mL	48.10
6	10 µg/mL	37.72

Figure 8 represents the antioxidant potential of ZnO NPs at various concentrations against the inhibition percentage using Ascorbic acid as the reference standard. It is evident that even at a low concentration of 10 µg/mL, ZnO NPs have around 41% antioxidant efficacy compared to ascorbic acid. The  $IC_{50}$  value was found to be 1.78 µg/mL.  $IC_{50}$  value is considered as a critical parameter for analyzing the antioxidant efficacy of a sample. It is defined as the amount of sample needed to reduce the concentration of DPPH by half of its initial value (Sánchez-Moreno et al. 1998). Thus, a very less value of 1.78 µg/mL clearly indicates that ZnO NPs are having excellent antioxidant properties.

The antioxidant activity of ZnO NPs has been explained by literature in several ways. It can be attributed to the development of stable DPPH molecule in the medium through electron density charge transfer phenomenon from the oxygen to nitrogen center of DPPH (Madan et al. 2016). Another possibility is the surface generation of electron–hole pairs in high quantity on ZnO NPs. This process leads to the creation of hydroxyl and hydrogen radicals through water splitting through the generation of high redox potential, which finally leads to the formation of a stable DPPH molecule (Sun et al. 2011; Kiran Kumar et al. 2014). All these results show the synthesized ZnO NPs possess a good extent of antioxidant activity.

## Conclusion

In this study, *S. dulcis* leaf extract was used to synthesize ZnO NPs through a simple and eco-friendly approach. *S. dulcis* is a common medicinal plant containing several phytochemicals responsible for biological activity, which serves as a bio-reductant for obtaining ZnO NPs from the precursor. XRD analysis showed that the average size of the ZnO NPs is approximately 20.2 nm. FTIR results showed that the phytochemical constituents of *S. dulcis* were responsible for the production of ZnO NPs and FESEM results revealed pebble-like morphology. The agar-well diffusion method was employed to evaluate various biological activity such

**Fig. 8** DPPH assay for antioxidant activity of ZnO NPs

as antibacterial, antifungal, and DPPH assay to measure antioxidant activity. ZnO NPs showed good antibacterial activity against both *E. coli* (gram-negative) and *S. aureus* (gram-positive) and antifungal activity against *C. albicans* and *A. niger* at 500 µg/mL. In addition, the excellent antioxidant activity of ZnO NPs were revealed by DPPH assay, which is evident from a very lower  $IC_{50}$  value of 1.78 µg/mL. These results indicate that the ZnO NPs synthesized through *S. dulcis* plant extract have the potential for employing in biomedical applications.

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## Declarations

**Conflict of interest** The authors report no declaration of interest.

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