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Biosynthesis of Zinc oxide Nanoparticles using *Barringtonia acutangula* Flower Extract and Evaluation of their Antibacterial and Antioxidant Activities

Namrata Jain[1], S.Chandra Mohan[2], S. Krishnaveni[3*]

[1,2]*Department of Chemistry, Sarvepalli Radhakrishnan University, NH-12, Hoshangabad Road, Jatkhedi, Bhopal- 462026, Madhya Pradesh, India*
[3]*Department of Chemistry, St. Joseph's College of Engineering and Technology , Elupatti, Thanjavur – 613 03, Tamil Nadu, India.*

***Corresponding Author: S**. Krishnaveni, Department of Chemistry, St. Joseph's College of Engineering and Technology , Elupatti, Thanjavur – 613 03, Tamil Nadu, India.*
Email: skvtnj12@gmail.com

Article History

Abstract

Zinc oxide (ZnO) has broad applications in various areas. Nanoparticle synthesis using plants is an alternative to conventional physical and chemical methods. It is known that the biological synthesis of nanoparticles is gaining importance due to its simplicity, eco-friendliness and extensive antimicrobial activity. Also, in this study we report the synthesis of ZnO nanoparticles (ZnONPs) using *Barringtonia acutangula* flower extract. The prepared ZnO nanoparticles have been characterized by UV–Vis absorption spectroscopy, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), and scanning electron microscopy (SEM)). The antioxidant activity of the nanoparticles was tested by the α-diphenyl-β-picrylhydrazyl (DPPH) method. Antibacterial properties of the synthesized ZnONPs were evaluated against *Staphylococcus aureus* and *Escherichia coli* by disc diffusion method,

***Key words: Barringtonia,* ZnO nano, antioxidant, antimicrobial, SEM**

INTRODUCTION

Nowadays, the green synthesis of metal nanoparticles is an interesting issue of nanoscience. Also, there is growing attention to the biosynthesis of metal nanoparticles using organisms. Among these organisms, plants seem to be the best candidates and they are suitable for the large-scale biosynthesis of nanoparticles. Nanoparticles produced by plants are more stable and more varied in shape and size in comparison with those produced by other organisms [1]. Metal oxides with nanostructure have attracted considerable interest in many areas of technology [2]. Among metal oxide nanoparticles, zinc oxide (ZnO) has received much attention in the recent past. ZnO nanostructures are the forefront of research due to their unique properties and wide applications [3]. There are different methods used for the synthesis of zinc oxide nanoparticles: direct precipitation, homogeneous precipitation, solvothermal method, sonochemical method, reverse micelles, sol gel method, hydrothermal, thermal decomposition, and microwave irradiation [4]. The biological method of the synthesis of ZnO nanoparticles is gaining importance due to its simplicity, eco-friendliness and extensive antimicrobial activity [5]. According to Mahanty *et.al.*, 2013 [6], the use of eco-friendly biosynthesized

nanoparticles as an alternative to the chemically synthesized ones would help control chemical toxicity in the environment. Divyapriya *et.al*., 2014 [7] synthesized ZnO nanoparticles using the ethanol extract of *Murraya Koenigii*. The results indicated that ZnO nanoparticles showed effective antibacterial activity against Gram negative and Gram positive bacteria. Gunalan et al., 2012 [5] showed that the green synthesized ZnO has a stronger inhibitory effect than chemically synthesized nanoparticles.

Barringtonia acutangula (L.) Gaertn. (Family: Lecythidaceae) known as Kadambu in Tamil; Samudraphal in Hindi, is a medium-sized tree found throughout India. *Barringtonia acutangula* is used as a folk medicine for curing various diseases like pain in joints, eye diseases, stomach disorders, diarrhea, cough, leprosy, and spleen disorders [8]. An aqueous extract of the bark is found hypoglycemic and is reported to be used in pneumonia, asthma and leaf juice is given for diarrhea [9]. A brief review of the work already done in the field. From the previous study, seven compounds have been identified from ethanolic extract of the stem bark of Barringtonia acutangula by Gas GC-MS analysis [10]. Kathirvel and Sujatha (2012) [11] investigated the antioxidant potential of *B.acutangula* leaves in various extracts. Previous studies indicated that triterpenoid derivatives were the main constituents of *B. acutangula* [12-14]. Steroidal compounds such as barringtogenic acid, tangulic and acutangulic acids were found in *B. acutangula* leaves [15]. Three flavan-3-ol derivatives and four flavonoid glycosides were isolated from the bark and leaves of *B. acutangula* [16,17]. The phytochemical analysis of *B.acutangula* showed the presence of flavonoids, glycosides, tannins, saponins, steroids, phenols, alkaloids. The present study reports for the first time green synthesis of ZnONPs by using *B.acutangula* flower extract and their antibacterial and antioxidant activities.

MATERIALS AND METHODS

Plant collection

Fresh and healthy flowers of *B.acutangula* were collected from Madurai forest area, Tamil Nadu (India).

Preparation of plant extracts

The flowers of *B.acutangula* were washed twice with distilled water and air-dried for 5 days at room temperature. To 5 g powder of *B.acutangula* flower, 200 ml of double distilled water was added. Then, the solution was stirred for 45 min in the temperature of 80 °C.

Synthesis of ZnO nanoparticles

ZnONPs were synthesized using zinc acetate dehydrate $Zn(CH_3COO)_2 \cdot 2H_2O$ as described previously [18]. Briefly, 90 ml of zinc acetate solution (0.01 M) was mixed with10 ml of plant extract and incubated overnight at room temperature. Finally, the prepared ZnONPs were separated by centrifugation of the reaction mixture at 4500 rpm for 15 min, followed by washing several times with deionized water and then drying at 80°C for 7–8 h [5,19].

Characterization of NPs

The synthesized ZnONPs were analyzed using a UV-visible spectrophotometer -1800. X-ray diffraction (XRD) analysis was performed on an X-ray diffractometer operated at 40 kV and 14 mA with scan speed/duration time 10.0000 deg/min, step width 0.0200 deg , scan axis Theta/2-Theta , scan range 3.0000 - 90.0000 deg was used to determine the crystallinity, purity, and size of the NPs. The surface morphology of ZnONPs was examined by a scanning electron microscope (SEM) operated at an accelerating voltage at 10 kV. The binding properties of ZnO nanoparticles using *B.acutangula* extract were investigated by FTIR analysis. The characterization involved Fourier transform infrared spectroscopy (FTIR) analysis of the dried powder of the synthesized ZnO nanoparticles by Perkin Elmer Spectrum 1000 spectrum in attenuated total reflection mode, and using the spectral range of 4000–400 cm^{-1}.

Evaluation of the antioxidant activity of ZnONPs with DPPH

The antioxidant activity of ZnONPs was evaluated on the basis of inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals [20]. One milliliter of ZnONPs at various concentrations (6.25, 12.5, 25, 50, and 100 µg/ml) and ascorbic acid (as a standard) were added to different test tubes. Next, 1 ml of DPPH (1mM) dissolved in methanol was added to each tube and vortexed thoroughly. Lastly, the solution was incubated at room temperature in dark for 30 min. The absorbance at 517 nm wavelength was read with a spectrophotometer, and the percentage of inhibition was calculated using the following equation:

$\%inhibition=[(Abs_{Control}-Abs_{Standard})/Abs_{Control}] \times 100$ ……(1)
where $Abs_{Control}$ is the absorbance of control and $Abs_{Standard}$ is the absorbance of sample. Ascorbic acid was used as the standard solution, while 1 mM DPPH was used as a control.

Antibacterial assay
The in-vitro screening for antimicrobial was carried out using selected pathogens which includes gram positive bacterium (*Staphylococcus aureus*) and two gram negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*). The 6mm (diameter) discs were prepared from Whatmann No. 1 filter paper. The discs were sterilized by autoclave at 121°C. After the sterilization, the moisture discs were dried in hot air oven at 50°C. Then various solvent extract discs and control discs were prepared. Antibacterial activity test was carried out following the modification of the method originally described by Bauer et al., (1966) [21]. Muller Hinton agar was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45ºC. The cooled media was poured onto sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The various solvents extract-prepared discs individually were placed on each petri plates and also placed control and standard (Nitrofurantoin (300 µg) for Bacteria) discs. The plates were incubated at 37ºC for 24 hrs. After incubation, the diameter of the zone formed around the paper disc was measured and expressed in mm. The antibacterial activities of ZnONPs were assessed against *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* .

RESULTS AND DISCUSSION

UV–Vis Spectra
The optical absorption spectra of ZnO dispersed in water were recorded using UV–Vis Spectrophotometer 1800. Fig. 1 shows the UV–Vis absorption spectrum of ZnO nanoparticles sample at different times. Typical exciton absorption at 393 nm was observed at room temperature. According to Gupta et al., 2014 [22], the absorption edge systematically shifts to the lower wavelength or higher energy with the decreasing size of the nanoparticle.

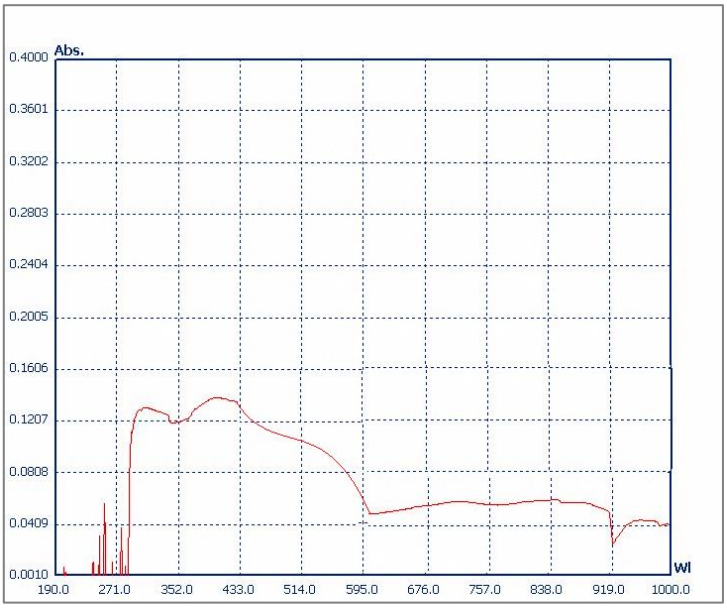

Fig.1. The UV–Vis absorption spectra for ZnO nanoparticles

Fourier transform infra-red spectroscopy (FT IR)
The results were further reinforced by FT-IR analysis, which showed the shifts and difference in areas of the peaks. FT-IR spectroscopy consists in measuring the absorption of IR radiations by a sample, and the results of such measurement are shown by means of a wavelength. The reading of the IR spectrum includes the interpretation of the interdependence between the absorption bands (vibrational bands) and the chemical compounds in the sample. By means of this technique, it is possible to identify the biomolecules in plant extracts which play the crucial role in the processes of reduction and stabilisation of the green synthesis of nanoparticles[23].

Fig. 2 shows the IR spectrum of the sample . According to Fig. 2, it is observed that the bands are at 3435 cm^{-1}, 2025 cm^{-1}, 1633 cm^{-1}, 1384 cm^{-1}, 1315 cm^{-1}, 1102 cm^{-1}, 779 cm^{-1} 601 cm^{-1} and 473 cm^{-1}. The FTIR spectrum of ZnO nanoparticles was recorded in the range of 400–4000 cm^{-1}. The peak in the region between 473 and 601 cm^{-1} is allotted to Zn–O [24]. Also, the band located near 515 cm^{-1} is assigned to ZnO stretching vibration. The bands at 3435 cm^{-1} and 1633 cm^{-1} are characteristic for hydroxyl group (O–H). The peaks at 1384 cm^{-1} and 1102 cm^{-11} may be ascribed to –C–O and –C–O–C stretching modes. The band which appeared at 2025 cm^{-1} is due to C=C stretching.

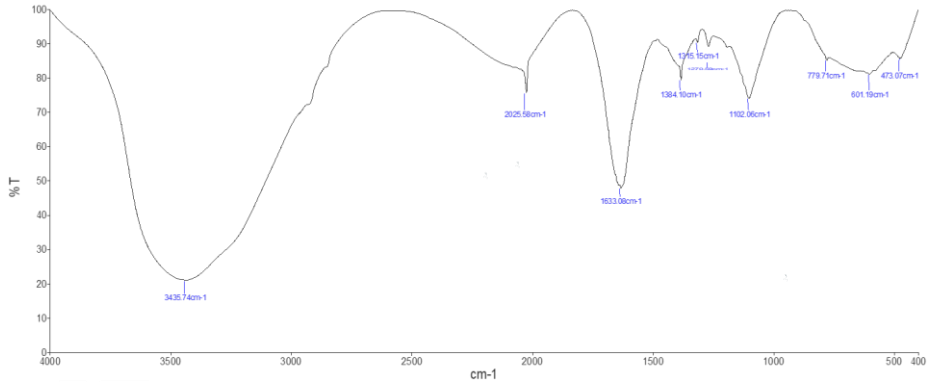

Fig.2: FT-IR spectra of synthesized ZnO nanoparticles using *B.acutangula* flower extract

Scanning electron microscopy (SEM)

The morphology of the nanostructures was studied using scanning electron microscopy (SEM). Fig.3 present the SEM images of the obtained ZnO nanoparticles. The synthesized ZnO nanoparticles were agglomerated with a particle size ranging from below 73–104 nm. The numbers Pa1, Pa2, Pa3 indicate the points in which the measurement was made. Additionally, the presence of highly pure ZnO is confirmed by X-ray diffraction XRD (Fig.4).

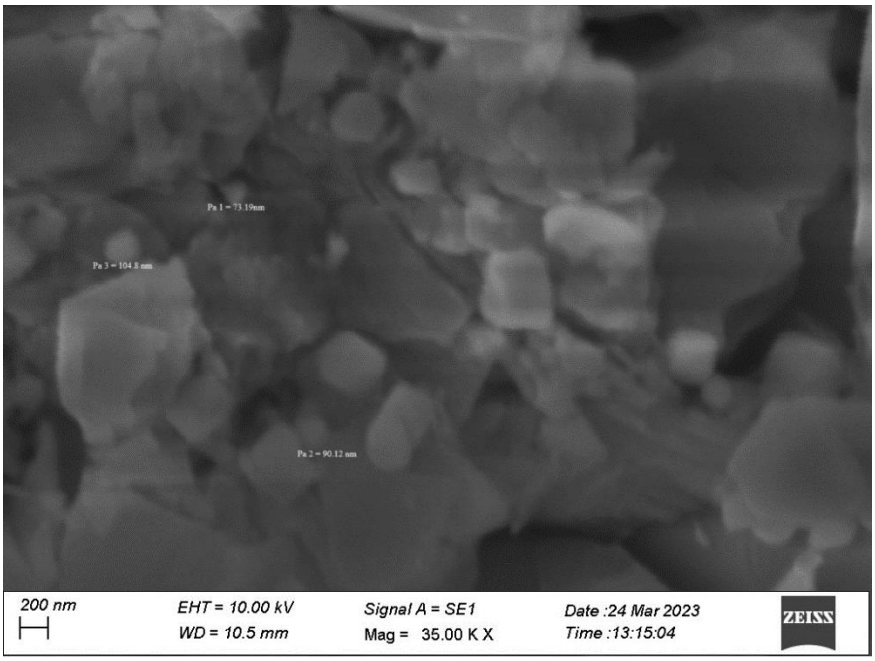

Fig.3: Scaning electron microscopy of ZnO nanoparticles synthesized using *B.acutangula* flower water extract

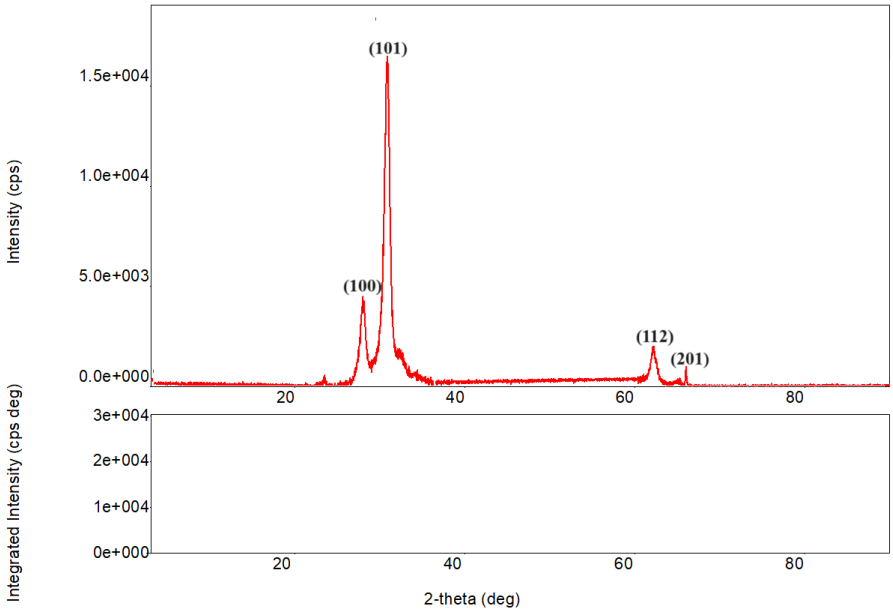

Fig.4 : XRD patterns of synthesized ZnO nanoparticles synthesized using *B.acutangula* flower extract.

The XRD pattern of the synthesized ZnONPs clearly indicated the crystalline structure of the synthesized NPs (Fig.4). The XRD pattern showed four distinct diffraction peaks at 30.93, 36.86, 65.92, and 67.20 degrees. These peaks were indexed as (100), (101), (112), and (201) diffraction lattice planes, respectively, which confirmed the hexagonal wurtzite structure of the synthesized NPs; this finding is in agreement previous study [25-27]. The average size of ZnONPs was calculated from the highest intense peak (101) using the Debye-Scherer equation:

$D=0.89\ \lambda/(\beta Cos\theta)$…… *(2)*

Where, D – the crystal size, λ – the wavelength of the X-ray radiation (λ = 0.15406 nm) for CuKα, β – the line width at half-maximum height.

The Scherrer formula was used to calculate the particle sizes and was found to be in the range of 80 nm. XRD study confirmed the presence of even smaller particles than the SEM examination. The larger nanoparticles of ZnO (more than 100 nm) in the sample result from the agglomeration of smaller nanoparticles, whose presence is confirmed by X-ray diffraction (XRD). The XRD method allowed for the identification of smaller sizes of nanoparticles. The agglomeration of smaller nanoparticles occurs due to the fact that we are dealing with biological material.

Antimicrobial activity

The antibacterial activity of ZnO nanoparticles was evaluated by measuring the zone of inhibition against the test organisms. The sizes of the zones of growth inhibition are presented in Table 1 and Fig.5. The results indicated that ZnO nanoparticles synthesized from *B.acutangula* flower extract showed effective antibacterial activity against all tested strains.

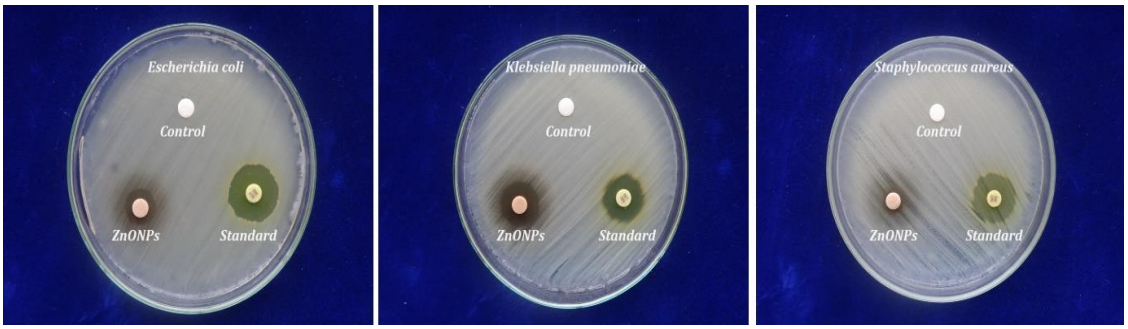

Fig.5: Antibacterial activities of ZnONPs were assessed against *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* compared with standard

Table 1. Antimicrobial activity of ZnO nanoparticles compared with standard

Microorganisms	Zones of growth inhibition [mm]		
	Control	**ZnONPs**	**Standard***
E. coli	-	14	20
K. pneumoniae	-	17	17
S. aureus	-	13	19

*Nitrofurantoin (300µg)

Mechanism of Antibacterial Activity of ZnO-NPs

Distinctive mechanisms that have been put forward in the literature are listed as following: direct contact of ZnO-NPs with cell walls, resulting in destructing bacterial cell integrity [28], liberation of antimicrobial ions mainly Zn^{2+} ions [29,30], and ROS formation [31,32]. According to Divya et al., 2013 [33], ZnO nanoparticles cause disruption of bacterial membranes probably by the production of reactive oxygen species, such as superoxide and hydroxyl radicals. Moreover, ZnO nanoparticles have positive zeta potential at their surface. This depends on the nature of the surface of different bacteria. Moreover, the antibacterial activity is reported to be dependent on the concentration of ZnO nanoparticles and the impact of the type of surfactant used. Also, ZnO nanoparticles could be attributed to the damage of the bacterial cell membrane and extrusion of the cytoplasmic contents thereby resulting in the death of the bacterium. On the basis of the research, it can be concluded that the inhibition of bacterial growth by ZnO nanoparticles could be attributed to the damage of the bacterial cell membrane and the extrusion of the cytoplasmic contents thereby resulting in the death of the bacterium [7].

Antioxidant activity of ZnONPs

ZnONPs showed effective inhibition activity in the DPPH scavenging assay as compared to the standard ascorbic acid (Fig.6).

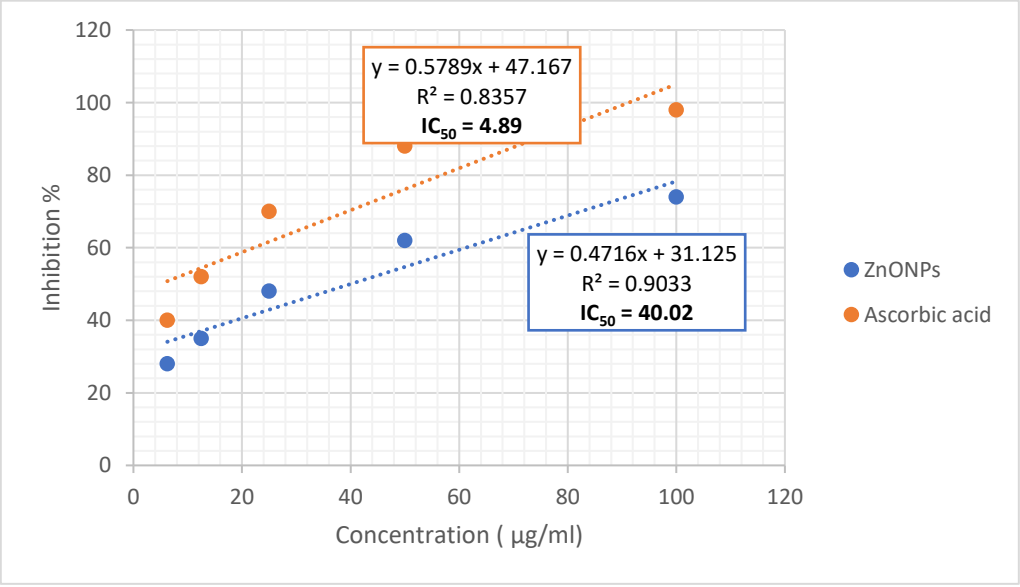

Fig.6: Antioxidant activity of synthesized ZnONPs compared with standard ascorbic acid

The significant antioxidant potential of ZnONPs was due to the hydrogen-donating ability of the active phytochemicals of *B.acutangula*, such as flavonoids, glycosides, tannins, saponins, steroids, phenols, and alkaloids that are capped over ZnONPs [34]. The DPPH activity of the ZnONPs was found to increase in a dose-dependent manner. When ZnONPs were added to the DPPH solution, a colour change occurred due to the scavenging of DPPH because of donation of a hydrogen atom to stabilize the DPPH molecule, which is responsible for the absorbance at 517 nm [35]. Previous studies support the antioxidant activity of ZnONPs [36]. The DPPH values increased in a dose-dependent manner. The radical scavenging capacity of the synthesized ZnONPs was slightly lower than that of the standard ascorbic acid at all the tested concentrations of NPs (Fig.6). The obtained results also revealed that the recorded IC_{50} (50% inhibition of free radicals) values of ZnONPs and ascorbic acid (used as a positive control) for DPPH radical inhibition were 40.02 µg/ml and 4.89 µg/ml, respectively. This finding suggests that the synthesized ZnONPs showed good antioxidant activity

as compared to ascorbic acid. Previously, ZnONPs synthesized by using an aqueous extract of *Coccinia abyssinica* exhibited the maximum DPPH scavenging activity at an IC_{50} value of 127.64 µg/ml [37].

CONCLUSION

It is known that the green synthesis of ZnO nanoparticles is much safer and environmentally friendly as compared to chemical synthesis. In response to this assumption, this study demonstrates the green synthesis of ZnO nanoparticles using *B.acutangula* flower water extract. The synthesized ZnO nanoparticles were characterized by UV–Vis absorption spectroscopy, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM). These methods confirmed the presence of the synthesized ZnO nanoparticles in the range of 80 nm. The larger nanoparticles of ZnO resulted from the agglomeration of smaller nanoparticles. Moreover, the synthesized ZnO nanoparticles exhibited high antimicrobial activity against *K. pneumoniae*. Also, the green synthesis of ZnO nanoparticles using *B.acutangula* flower extract can be an alternative to chemical methods.

Conflict of interest

The authors declare that they have no conflict of interest.

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