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Original Research

Potential Antifungal and Antimicrobial Effects of Nano Zinc Oxide Particles Obtained from *Cymbogobon citratus* Leaf Extract Using Green Technology

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Abstract

Nanoparticles obtained from physical and chemical methods are uniformly sized having long term stabilities. However, their syntheses usually involve expensive chemicals which are toxic, carcinogenic and require high temperature and pressure. These issues can be addressed by facile and eco-friendly green synthesis. This study focuses on to synthesis of zinc oxide nanoparticles (ZnO NPs) using *Cymbogobon Citratus* aqueous extract with zinc acetate. The prepared particles were calcined at 450°C. The synthesized NPs were categorized using FTIR, UV-Visible, XRD and SEM techniques. FTIR verified zinc oxide vibrational frequency at 551 cm^{-1}. UV-Visible spectrum displayed the ZnO absorption peak at λ_{max} of 370 nm. XRD studies showed average particle size about 4.96 nm much smaller as compared to most of the ZnO NPs reported earlier. SEM image demonstrated crystalline and porous nano-sheets for the prepared NPs. These NPs were also evaluated against *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Microsporum cannis, Aspergillus flavus* and *Fusarium*

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solani. The synthesized ZnO NPs exhibited highest antibacterial and antifungal potential against *S. Aureus* and *A. flavus*, respectively.

Keywords: antifungal, bioremediation, plant mediated, antibacterial, metal nanoparticles

Introduction

Nanoparticles have tremendous applications in the field of medicine. NPs are used in the regeneration of various tissues/organs, in targeted drug delivery and cell bio-imaging, for treatment of cancer, neurodegenerative diseases, inflammatory diseases, cardiovascular and pulmonary diseases, atherosclerosis, Parkinson's disease, human immunodeficiency virus, tuberculosis, skin infections and bone inflammation [1-5]. Many nanotechnology-based products have been authorized as therapeutic agents in the past few decades. Nanoparticles also find their uses in packaging, catalysis, electronics, photocatalysis, materials for photovoltaic cells, fuel cell, mechanics and cosmetics and also for the reduction of environmental contamination and global warming by using as catalysts [6-9].

Zinc oxide finds significant industrial importance due to its versatile properties, which can be improved by producing this material on the nanoscale. Zn/ZnO NPs are being employed as optical, photocatalytic, antioxidant, biomedical, cytotoxic, antimicrobial, antibacterial, anticancer and antidiabetic materials [10-12]. They show good potential against resistant and pathogenic infections caused by bacteria and show prophylactic effects on chronic toxoplasmosis in mice. The NPs were biocompatible with human erythrocytes. ZnO NPs can be applied on fresh strawberries as coatings for their preservation [13]. In agricultural field, foliar application of zinc NPs as fertilizers and as nano-nutrients can improve plant growth, root and seed germination. They are very vital for the enhancement of nutrients uptake for many plants e.g. they show exceptional antimicrobial and agricultural efficacy in maize.

The NPs can possibly be synthesized through physical, chemical, biological and green methods. Green route is nontoxic, ecofriendly, clean, cheap and safe. Phytochemicals derived from plant leaves are very vital for production of NPs of various sizes and yields. Since the chemical composition of leaves varies from plant to plant so extent of NPs formation also varies according to the nature of plant used in biosynthesis. Green synthesis of Zn/ZnO NPs was reported in earlier literature yet no study was reported with C. *citratus*. This plant is commonly found worldwide and finds a tremendous medicinal and nutritional value [14-16].

Currents studies were performed to investigate the synthesis of ZnO NPs in lemongrass extracts. The synthesized NPs were characterized by sophisticated techniques. They were also subjected to evaluation of their antimicrobial potential and antifungal potential against pathogens.

Materials and Methods

All the reagents and chemicals used in this scientific work were of high purity. The fresh young leaves of *Cymbogobon citratus* at the time of flowering were collected from Baagh-e-Jinnah, Lahore, Pakistan. The leaves were comprehensively washed with distilled water, dried under the shade for a week at room temperature and finally ground by a grinder to obtain the fine powder. The FTIR spectroscopy of the synthesized nanoparticles was performed by IR tracer-100 Shimadzu spectrophotometer. The XRD analysis was performed by X-ray diffractometer D8 Discoverer Bruker, Germany. For SEM analysis, scanning electron microscope EVO LS10, ZEISS Germany was used.

Synthesized NPs were verified for their antimicrobial potential by agar well diffusion process. The aqueous solutions having 10 mg/mL, 50 mg/mL and 100 mg/mL concentrations of Zn-O NPs were used for the tests. The activity of NPs was appraised against *E. coli*, *S. aureus, S. Typhi, M. canis, A. flavus and F.solani* strains. Norfloxacin and Amphotericin-B were used as a Standard drug for antibacterial and antifungal activity evaluations. The zones of inhibition (ZOI) and MIC values were measured. Each bacterium was cultured in nutrient agar medium which was prepared by mixing distilled water (100 mL) and nutrient agar (2.5 g) on a hot plate and then sterilizing the mixture through autoclaving at 121ºC for 15 min. The bacteria cultured in medium were used for preparing inoculums.

The sterilized nutrient agar medium (30 mL) was poured in a glass petri plate and was placed for 24 h at room temperature. Then sterilized swab was used to spread 5 µL of each bacterium culture on a separate plate. 10 µL solution of calcined nanoparticles was added into three wells each. After 24 h, the ZOI were noted, which demonstrate the antimicrobial activity of sample.

Synthesis of ZnO Nanoparticles

Dehydrated (12 g) leaves were placed into 100 mL of de-ionized water and then this mixture was continuously stirred at 70ºC for 2 hrs. This mixture was cooled at 25ºC followed by filtration to obtain the aqueous extract (76 mL) of *C. citratus* leaves. Then this 50 mL of plant extract was mixed with 50 mL of 0.25 M zinc acetate in a 250 mL flask. The resultant mixture was left for 1 hour at 25ºC in order to settle down the precipitates of ZnO NPs which were separated by centrifugation at 4000 rpm and then dried. The obtained NPs were dried by placing them in an oven at 40ºC for 24 h, followed by calcination at 450ºC for 1 hour. Fig. 1 displayed schematic diagram for the formation of ZnO NP.

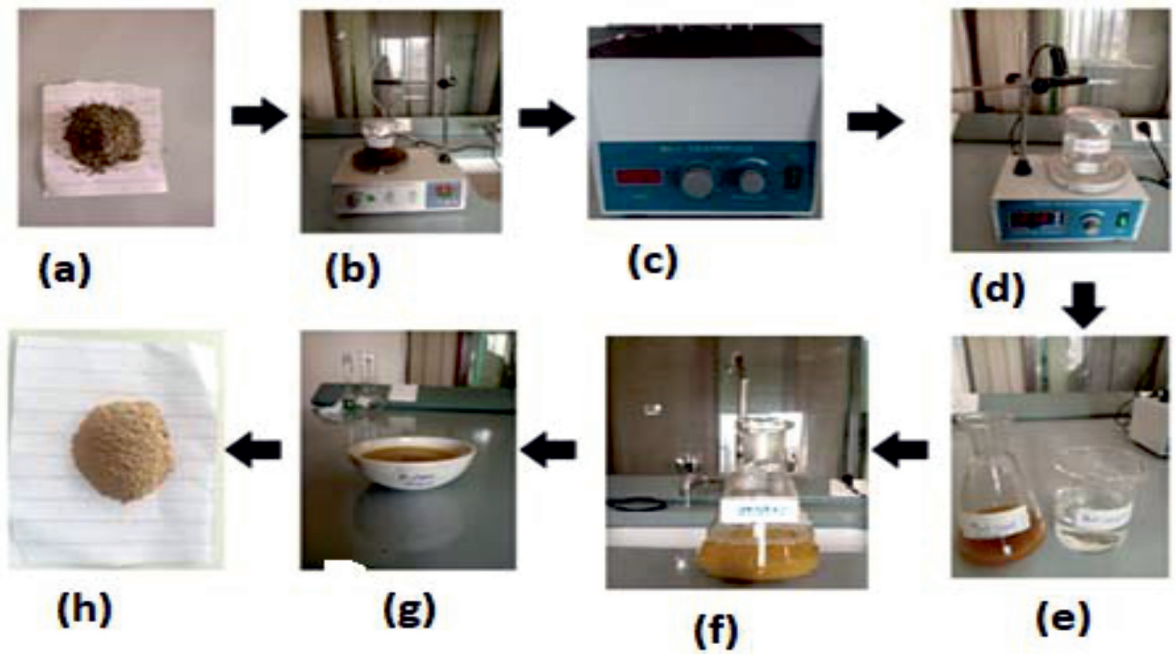

Fig. 1. Schematic diagram represents preparation of ZnO NPs from the lemongrass a) dried leaves powder of *Cymbopogon citratus* b) leaves extract (plant) c) centrifugation of extract d) salt solution e) plant extract with salt solution f) settle down zinc oxide NPs having supernatant layer g) oven dried ZnO NPs at 60ºC temperature.

Results and Discussion

The synthesized ZnO NPs were categorized by highly sophisticated analytical techniques. Furthermore, the prepared NPs were also tested against bacterial strains and fungal strains.

FTIR Spectroscopy

The vibrational spectrum of NPs was recorded from 500-4000 cm^{-1}. Fig. 2 shows the IR spectrum of the green synthesized ZnO NPs. It reports the presence of various functional groups and their significance. The FTIR spectrum displayed a large number of peaks, most of which were corresponding to the phytochemicals/ biomolecules which were involved in the reduction and stability of ZnO NPs.

The peaks at 3564, 2309, 1543, 1446, 1226 and 1145 cm^{-1} were assigned to N-H (stretch), S-H, N-H (bend), C-N, C-O stretch (phenolic) and C-O stretch (primary alcohol), respectively and can be owed to the functional moieties of plant extracts. The appearance of two bands at 756 cm^{-1} and 690 cm^{-1} demonstrates out of plane (OOP) bending vibrations of aromatic C-H bonds. The presence of a vibrational peak at 1029 cm^{-1} can be attributed to the presence of C=S moiety [17]. However, the peak of Zn-O has a special importance, which appeared at 551 cm^{-1} in the spectrum.

UV-Visible Spectroscopy

Green synthesized ZnO NPs were analyzed for electronic excitations in the range of 200-800 nm. A 100 mg/mL solution of NPs was used to record the UV-visible spectrum (Fig. 2). The spectrum has shown a characteristic absorption at 370 nm (λmax = 0.7) which establishes an inherent band-gap absorption of ZnO due to the electronic transitions ($O_{2p} \rightarrow Zn_{3d}$) and the presence of hexagonal wurtzite ZnO NPs according to reported literature [18 Wooten, 2009 #1630 Wooten, 2009 #1630]. Moreover, the sharp absorption (sharp peak) of ZnO NPs is an indication of nano-sized, narrow particle size distribution and monodispersed nature of the nanoparticle distribution [19, 20].

XRD Analysis

The crystallinity, index, nature, and size of green produced ZnO NPs were all measured using the AXS D8 X-ray diffractometer (XRD) analysis as shown in Fig. 2; the obtained and calculated data are shown in Table 1. The XRD peaks at 2θ angle are 31.83°, 34.52°, 36.29°, 47.58°, 56.65°, 62.89°, 66.41°, 67.93°, 69.18° and 77.01° correspond to the (100), (002), (101), (102), (110), (103), (200), (112), (201) and (202) Miller indexes planes respectively. The Scherer equation was employed for the determination of average particle size of the NPs;

$$D = \frac{K\lambda}{\beta \cos\theta}$$

where D stands for particles diameter, K represents the Scherer constant (0.9), λ is 1.5406 nm, β is full width at half maximum of the diffraction peak and θ is the

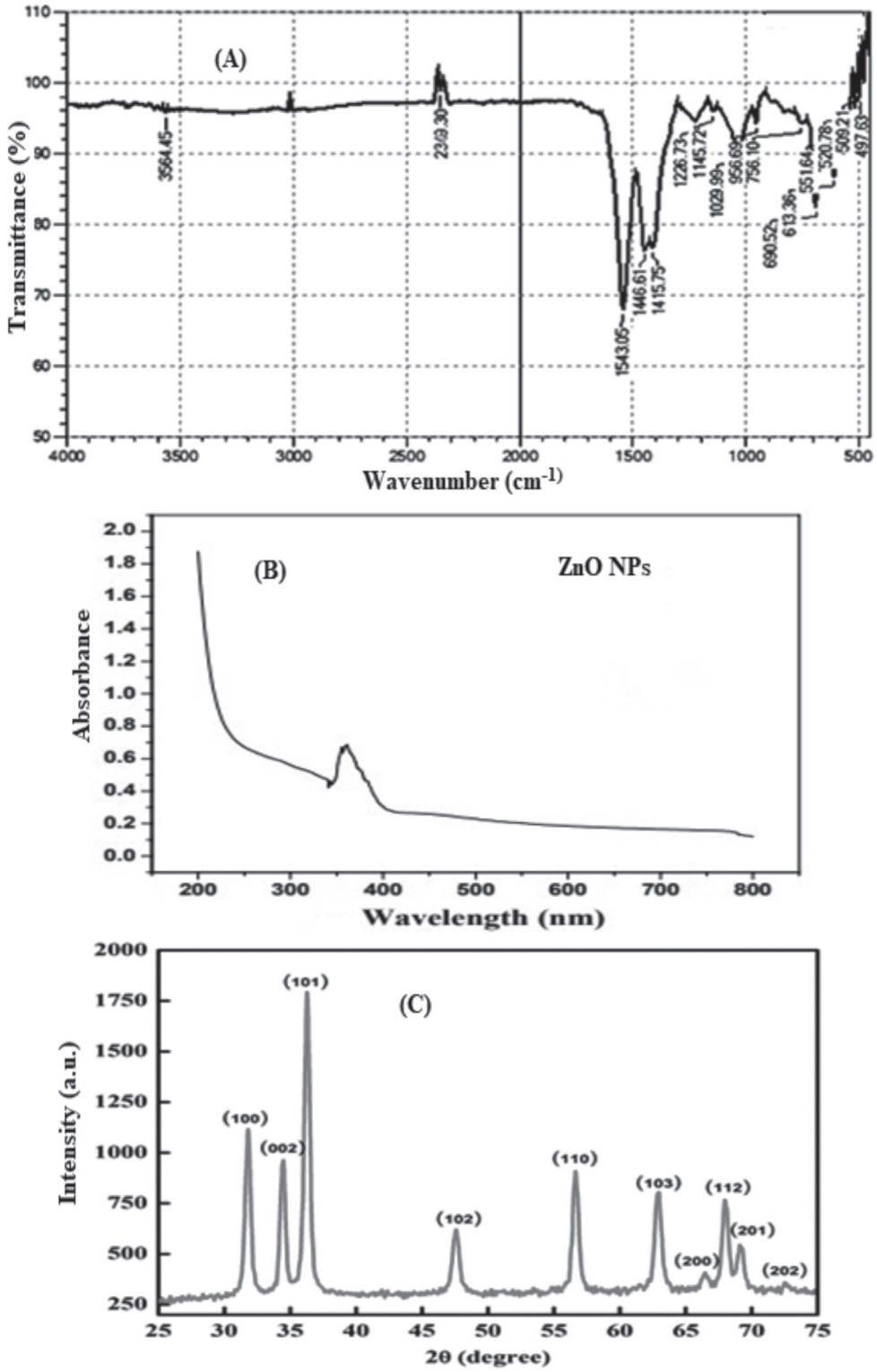

Fig. 2. Green synthesized ZnO NPs A) FTIR, B) UV-Visible and C) XRD of ZnO NPs.

angle of diffraction. The calculated average grain size of ZnO NPs particles from XRD was 4.96 nm (Table 1). The obtained crystallite size was significantly smaller (4.96 nm) as compared to that (25-100 nm) of most of the early reported ZnO NPs which were produced by using different plant materials.

SEM Analysis

A field emission scanning electron microscope (SEM) (HITECH-3400-N) was used to represent the morphology of synthesized zinc oxide nanoparticles. It was found that ZnO NPs are produced in crystalline form and have porous sponge like nano-sheets

Table 1. Crystallographic parameters and Miller Indices for the synthesized nanoparticles.

2Ø (degree)	$Sin^2Ø$	1×$sin^2Ø$/ $Sin^2Ø$min	2×$Sin^2Ø$/ $Sin^2Ø$min	3×$Sin^2Ø$/ $Sin^2Ø$min	Miller Indices
31.8355	0.0752	1.1512	2.1142	2.5445	100
34.5207	0.0880	1.1702	2.3404	3.5106	002
36.2871	0.0969	1.2885	2.577	3.8665	101
47.5802	0.1627	1.848	3.696	5.543	102
56.6487	0.4744	6.308	12.616	18.924	110
62.8917	0.5216	7.206	14.412	43.236	103
67.9331	0.312	4.148	8.296	12.444	112
Material Characterization					
2Ø (degree)	FWHM (β)	d-spacing $d = λ/2Sin^2Ø$	Grain size (D)	Dislocation density (Ɣ)	Strain (S)
31.8355	0.28673	2.8102	5.029	0.0994	0.0689
34.5207	0.22556	2.5961	6.439	0.0776	0.0538
36.2871	0.29529	2.4742	4.944	0.1011	0.0701
47.5807	0.29426	1.9095	5.150	0.0970	0.0663
56.487	0.35102	1.6239	4.487	0.1119	0.0772
62.8917	0.35766	1.4759	4.545	0.1099	0.2348
67.9331	0.40304	1.3789	4.148	0.1205	0.0835
Average grain size of ZnO NPs			4.96 nm		

(Fig. 3) which were formed by numerous phytochemicals present in leaves of lemongrass. The current findings of nanosheet formation are in good agreement with those reported elsewhere.

Antimicrobial Activities

Synthesized ZnO NPs were tested for their antibacterial, antifungal potential using agar well diffusion method as well as minimum inhibitory concentration evaluations. The synthesized nanoparticles have shown lesser antimicrobial activities as compared to the standard antibacterial and antifungal drugs i.e. Norfloxacin and Amphotericin-B, respectively. The highest antibacterial and antifungal potential of the ZnO nanoparticles was displayed against *S. Aureus* and *A. flavus*, respectively so, the synthesized NPs may effectively be used against these microbial strains. The lowest antibacterial and antifungal activities were exhibited against *E. Coli* and *M. canis*, respectively.

Table 2. Antibacterial and antifungal activity data with zone of inhibition and minimum inhibitory concentration.

		Inhibition zones (mm)		MIC data (ug/mL)	
Antibacterial activities	Bacteria	ZnO NPs	Norfloxacin	ZnO NPs	Norfloxacin
	E. Coli	10	15	25	5
	S. Aureus	14	20	100	10
	S. Typhi	13	20	55	10
Antifungal activities	Fungai	ZnO NPs	Norfloxacin	ZnO NPs	Norfloxacin
	M. canis	12	30	65	20
	A. flavus	18	30	90	15
	F. solani	15	25	75	18

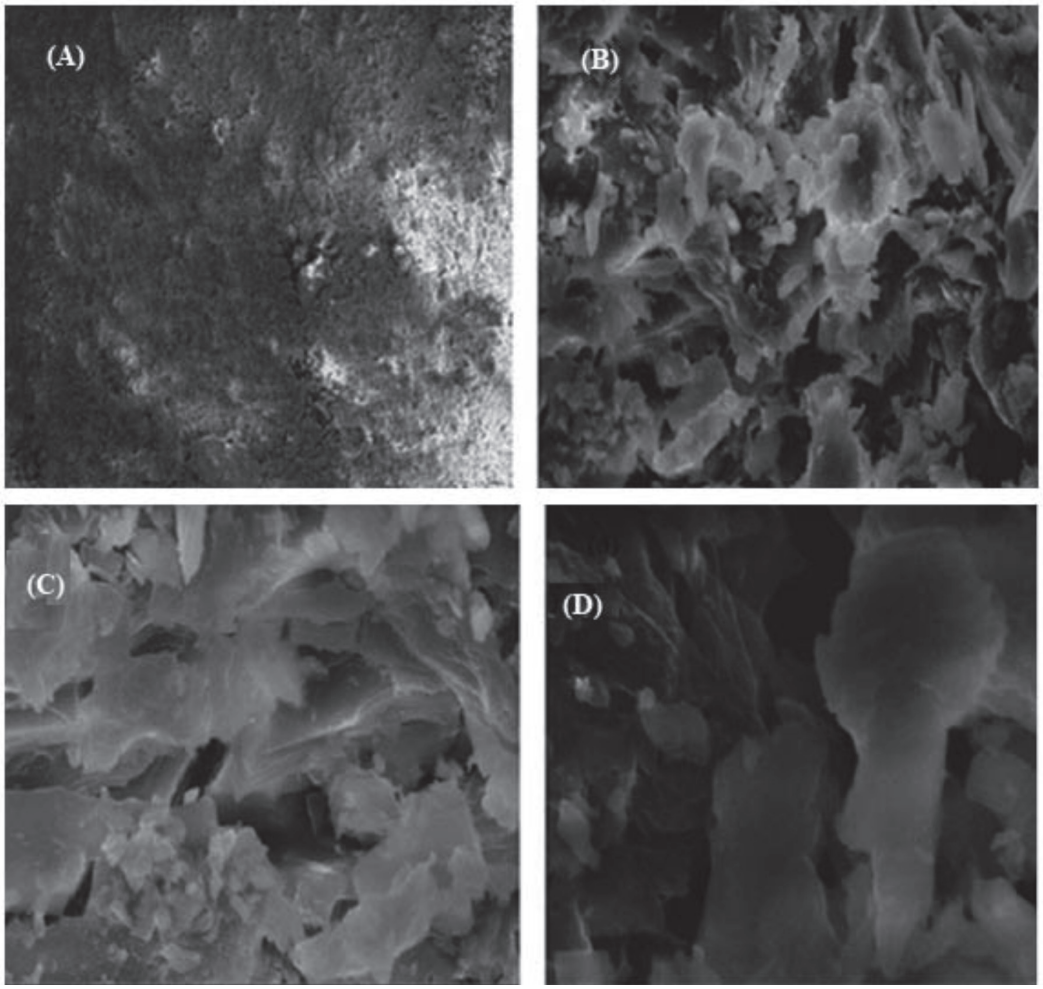

Fig. 3. SEM images of synthesized ZnO NPs from the lemongrass plant a) Porous; (b-d) Nano-sheet like structures.

Comparison of Results with the Previous Studies

The plant mediated synthesis of zinc oxide NPs was reported earlier by using various plant extracts including *Aloe barbadensis* leaves, *Pongamia pinnata* leaves, *Solanum torvum* leaves, *Salvia officinalis* leaves, *Lycopersicon esculentum* (tomato) juice, *Lycopersicon esculentum* (tomato) juice, *Elaeagnus angustifolia* L. leaves, *Azadirachta indica* leaves, *Phyllanthus niruri* leaves, *Agathosma betulina* leaves and dried rhizome of *Coptidis Rhizoma* [21-23]. The earlier reported ZnO NPs possessed the crystallite sizes of 2.9-100 nm and were produced in various forms including spherical (most common form), quasi-spherical, hexagonal wurtzite and agglomerate form. These NPs were investigated for antibacterial, antifungal, toxicological, biomedical & in-*vitro* biological activities, for cosmetic, photovoltaic, photocatalytic, catalytic, pyroelectric, optoelectronic and piezoelectric applications. However, we have produced the ZnO NPs in the current study by using the aqueous extract of *C. citratus* leaves. The synthesized NPs had shown the crystallite sizes of 4.96 nm with the porous crystalline nanosheets. They have shown significant potential against the tested bacterial (*S. Aureus*, *S. typhi* and *E. coli*) and fungal (*A. flavus*, *M. cannis* and *F. solani*) strains.

Conclusions

ZnO NPs were synthesized through plant mediated synthesis. The *C. citratus* leaves were used for the synthesis purpose. The synthesized nanoparticles were characterized by FTIR, UV-visible, XRD and SEM analyses. Antibacterial and antifungal studies were performed using the prepared nanoparticles. FTIR spectroscopy displayed a characteristic Zn-O vibration and other peaks for the functional groups of phytochemicals in leave extracts. The purity, structure size and nature of synthesized NPs were verified by X-ray diffraction patterns. The average grain size was found to be 4.96 nm which was smaller as compared to that of the mostly reported ZnO NPs. SEM analysis demonstrated the porous crystalline nano-sheets of the synthesized NPs. The NPs have shown significant potential against the tested bacterial and fungal strains.

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Conflict of Interest

The authors declare no conflict of interest.

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