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Greener synthesis of zinc oxide nanostructures using selected amino acids for assessment of antibacterial activity

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Abstract: **Background**: This study presents a simple and eco-friendly green synthesis approach for the preparation of amino acid–assisted zinc oxide nanostructures (ZnONSs). **Objectives**: The objectives of synthesizing amino acid-assisted ZnONSs were to evaluate their antibacterial applications. **Methods**: Among the amino acids selected are Arginine, aspartic acid, cystine, and lysine. These amino acids were used as capping and stabilizing agents to tailor the structural and surface properties of ZnONSs. The synthesized nanostructures were characterized using X-ray diffraction (XRD), UV–Visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDS) and disk diffusion method for antibacterial activity analysis. **Results**: XRD analysis confirmed the formation of ZnO with altered crystallinity due to amino acid incorporation, while UV–Vis spectroscopy verified successful synthesis of ZnONSs. FTIR spectra demonstrated effective surface functionalization by amino acid–related functional groups, and SEM revealed amino acid-dependent morphological variations, including rod- and flower-like structures with increased surface area. EDS further verified the predominance of Zn in the samples. The disk diffusion study confirmed effective antibacterial activity against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative), with performance varying according to the amino acid used during synthesis. Among the samples, arginine-assisted ZnONSs demonstrated superior antibacterial efficacy, likely due to enhanced surface functionalization and electrostatic attraction with negatively charged bacterial cell membranes. **Conclusion**: This work highlights amino acid-assisted green synthesis as a promising route for developing eco-friendly ZnO-based antibacterial nanomaterials.

Keywords: Amino acids; Arginine; Aspartic acid; Antibacterial activity; Cysteine; Lysine; Zinc oxide nanostructures

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INTRODUCTION

Nanotechnology has been playing a pivotal role in this fast-growing world. Specifically, the materials science and engineering has a great impact through nanotechnology in terms of materials preparation, characterizations and their respective applications (Mladenova, Slavova *et al*., 2023). Nanotechnology deals with the nanomaterials ranging from 1-100 nm. Sufficient research has already been conducted in nanotechnology with different materials for various applications such as energy storage, conversion and biomedical, aerospace, water treatment and so on. Among all other applications of nanotechnology, synthesis of metal oxide nanostructures using amino acids as capping and stabilizing agents and their application as antibacterial analysis has also been extensively studied in the literature (Pandit, Roy *et al*., 2022). Amino acids are not only utilized as structural building blocks of proteins and biomolecules, but also observed to be used as an energy source in human body (Ling, Jiang *et al*., 2023). Metals are reported to possess antibacterial activity themselves. Metal oxides and metal oxide nanostructures also possess antibacterial activity against various species of bacteria. It is also reported that amino acids possess antibacterial activity. It was suggested that amino acid assisted metal oxide nanostructures may enhance antibacterial activity and will have synergistic effect (Panahi Chegini, Nikokar *et al*., 2019). Nanotechnology has been utilized across various fields, i.e., chemistry, biology, and physics. (Bhushan, 2017, Jaskulski, Jaskulska *et al*., 2022). In addition to metal oxides, ZnO nanostructures (ZnONSs) are being synthesized and applied in many fields such as biological, chemical, electrical, optical, antibacterial, sensors, semiconductors and piezoelectric devices, accordingly (Fang, Xu *et al*., 2025).

Literature surveys showed that many research scholars have synthesized ZnO nanoparticles and nanostructures using different methodologies. ZnO has been reported in different surface morphologies and shapes. The chemical and physical properties of such synthesized nano materials with their application in multiple fields were also covered broadly (Shan, Yang *et al*., 2021). Due to the distinct properties of ZnONSs, possible applications in almost every field, such as biosensors, nanomedicine and nanotechnology, have been observed. The basic properties of ZnONSs are characterized by their composition, size, crystal nature and shape, etc. (Dey, Mohanty *et al*., 2025).

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Moreover, ZnONSs have been observed recently along with significant status in the applied and basic sciences in field of bio-nanotechnology (Jha, Rani *et al*., 2023). Antibacterial activity studies of ZnOs are currently being examined using different formulations. When ZnO has been reduced in size (i.e. nanoscale), it shows antimicrobial activity. From this study, it can be assumed that ZnO at nano size can come in contact with surface of bacteria (at bacterial core). Through this core, ZnO can enter a bacterial cell, leading to bactericidal mechanisms (Saleh and Hassan, 2023). The interactions between ZnO and different species of bacteria are seen to be toxic for bacterial growth. ZnONSs are not harmful to human cells, which is why these metal oxide nanostructures were selected for this study (Colon, 2006). Also, it is reported that ZnONSs can enhance the antimicrobial or antibacterial activity against different species. It can help destroy the pathogen microorganisms with very good compatibility with human body cells *in-vivo* and *in-vitro* (Padmavathy, 2008; Wang, Xu *et al*., 2024).

Antibacterial response can be enhanced due to an increase in surface area of the nanomaterials and presence of some particular chemical substances with nanomaterial's may increase their permeability inside bacterial cell, which can help increase their functionality (Hui, Lai-Fa *et al*., 2024; Seil, 2009). Different investigations of antibacterial study of nanomaterials, mostly ZnONSs, with other materials such as amino acids can enhance the research area of nanomaterials as well as the mechanisms and phenomena behind nanostructured materials. Bacterial infections are serious problems that act as a human health threat, which extends on many complications. Increased outbreaks and infections of pathogenic strains, bacterial antibiotic resistance, emergence of new bacterial mutations, lack of proper usage of antibiotics in underdeveloped countries and hospital-associated infections are global health hazards to humans, particularly in children (Gao, Wang *et al*., 2025, Mendes, Dilarri *et al*., 2022, Sobhy, 2024). Thus, developing new antibacterial agents against bacteria, mostly major food pathogens, has become in demand. This work is intended to explore these problems to induce further investigations in these areas by addressing new techniques, benefiting from the unique features of ZnONSs with other molecules and to provide effective studies to date. In this paper, a new strategy has been developed for synthesis of ZnONSs using various amino acids. This is one of the greener ways to synthesize ZnONSs. Herein, Arginine (Arg), Aspartic acid (Asp), Cysteine (Cys) and Lysine (Lys) have been used to synthesize ZnONSs. The selected amino acids (Arg, Asp, Cys, and Lys) offer a diverse range of functional groups-basic, acidic, and polar uncharged- allowing systematic tuning of ZnO nanostructure morphology, surface charge and defect states. Their biomedical relevance lies in enhancing bacterial membrane interactions and providing stabilization or Reactive Oxygen Species--related effects, resulting in a broad-spectrum antimicrobial strategy. Additionally, their biocompatibility and non-toxicity make them ideal candidates for eco-friendly, green synthesis of ZnO nanomaterials (Gao, Sun *et al*., 2025; Mahakal, Pathan *et al*., 2023; Sherine, Indubala *et al*., 2021). Different characterization techniques have been utilized for confirmation of shape, crystallinity, functional groups and chemical composition of synthesized ZnONSs. The antibacterial activity of the synthesized ZnO nanostructures (ZnONSs) was evaluated against two representative bacterial strains, namely *S. aureus* (Gram-positive) and E. coli (Gram-negative). The results demonstrated promising antibacterial potential, as the ZnONSs effectively inhibited the growth of both bacterial species.

MATERIALS AND METHODS

Chemical and reagents

All the chemicals used in this study were of analytical grade. Zinc acetate dihydrate, Urea, Arginine, Cystine, Aspartic acid and Lysine were purchased from Sigma-Aldrich Pvt. Ltd. Double distilled deionized water was used for all of the mentioned procedures.

Equipments

The XRD analysis was performed to examine crystal nature of ZnONSs. It was carried out via Bruker powder diffractogram having model No. D-8. UV-Vis absorption spectra were performed at PerkinElmer model Lambda 365. The functional group of nanostructures was analyzed using FTIR spectrometer model Spectrum Two FT-IR Spectrometer made by PerkinElmer. The morphology and elemental analysis of nanostructures were studied by SEM, and EDS was performed using a JEOL model number of SEM 6380-L.

Synthesis of amino acid assisted ZnONSs

Amino acids assisted with zinc oxide nanostructures have been synthesized by dissolving zinc acetate dihydrate (0.1M), urea (0.1M) in 250mL of DI water. Then different types of amino acids, such as Arginine, Aspartic acid, Cysteine and Lysine, were added in 1 and 2 mM concentrations individually and followed by continuous stirring. The precipitation was carried out at 70 ºC for 6 h for all prepared solutions. The obtained precipitates were washed with the help of DI water and filtered to collect the precipitates. After that, it was placed into preheated oven at 95 °C for 6 h for drying. The dried samples were placed in muffle furnace at 200°C for 4 h for calcination to form oxides. For the synthesis of pristine ZnO, a similar method was used without the addition of amino acid. All synthesized samples were given codes for quicker identification, given in table 1.

Physicochemical characterizations

The XRD analysis was performed to examine crystal nature of ZnONSs. It was carried out via Bruker powder diffractogram having model No. D-8. The operating conditions were CuKα radiation (λ = 1.5418 Å) with a

voltage of the generator of 45 kV and a current equal to 45 mA, respectively. UV-Vis absorption spectra (PerkinElmer: Lambda 365) were used to obtain the absorption spectra of nanostructures and calculate the energy band gap. FTIR was used to receive the surface functionalized data. The SEM (JEOL model number of SEM 6380-L) was used to characterize the morphology of synthesized ZnONSs using amino acids. The operating voltage of 20 kV was used for analysis. Energy dispersive spectroscopy (EDS) attached to SEM was applied to quantify the elements of the prepared nanostructures.

Antibacterial activity

After synthesis and characterization, the synthesized amino acid assisted ZnONSs were investigated for antibacterial activity against two bacterial species. These include one Gram-positive bacterium, i.e., S. aureus, and a Gram-negative bacterium, i.e., E. coli. The method adopted to check antibacterial activity was agar well diffusion. Agar medium (Mueller-Hinton agar) was prepared for antibiotic susceptibility testing. The agar was then allowed to solidify by leaving the plates uncovered on a clean surface. Bacterial cultures of E. coli and S. aureus were prepared from stock cultures. Once the agar was solidified, a sterile swab was used to streak the bacterial culture (*S. aureus* for gram-positive and E. coli for gram-negative) onto separate Mueller-Hinton agar plates and special care was taken to ensure even distribution across the entire surface. The prepared nanostructure suspensions were applied onto sterile paper discs and placed on the surface of the inoculated agar plates. Antibiotic discs (ampicillin) were also placed onto separate sections of the agar plates as positive controls for comparison. After that, the plates were incubated overnight at the appropriate temperature (usually 37°C) to allow bacterial growth and nanoparticle diffusion. After incubation, the diameter of the zones of inhibition (clear zones around the discs) was measured using vernier caliper. All experiments were conducted in triplicate and data are presented as mean ± standard deviation. (Bouchekrit, Laouer *et al*., 2016, Bughio, Bhatti *et al*., 2024).

RESULTS

The results of XRD, SEM, FTIR, UV-Vis spectroscopy and EDS were analyzed for prepared samples. The XRD pattern of pristine ZnONS and amino acid assisted ZnONSs is given in fig. 1. The diffraction patterns of ZnONS and amino acid assisted ZnONSs showed a hexagonal phase of ZnO in all samples, validated with the standard JCPDS card number (01-079-0208). In case of pristine ZnO, the peaks at 62.64, 56.31, 47.37, 36.10, 34.34, and 31.62° correspond to (103), (110), (102), (101), (002) and (100) diffraction planes, respectively.

In the range of 200-800 nm, the UV-Vis spectroscopy analysis of ZnONSs was carried out. The UV-Vis spectrum is presented in fig.2. In between 350 and 365 nm, the strong absorption peak for pristine sample was found. When amino acids were analyzed using UV-Vis spectroscopy, the characteristic peak of ZnO was diminished, which corresponds to the addition of amino acids on the surface of ZnO. The Tauc plot was used to determine the band gap energy of both pristine ZnO and amino acid assisted ZnONSs.

The functional group of pristine and amino acid assisted ZnONSs was analyzed using FTIR spectroscopy. The FTIR spectra of prepared samples are shown in fig. 3. The FTIR peak obtained at position of 3420 cm^{-1} is characteristic peak of –OH stretching vibration. While vibration bands obtained at 465 cm^{-1}, 571 cm^{-1} and 1022 cm^{-1} can be attributed to ZnO. In addition, FTIR band peaks present at 778 cm^{-1} and 885 cm^{-1} wavenumbers are assigned to C=C band, while the peaks observed at 2960 cm^{-1} and 2930 cm^{-1} are characteristic of C-H bond. In amino acids, some extra peaks were also observed.

The morphology of the pristine and amino acid assisted ZnONSs was studied using SEM. The microscopic images of nanostructures have revealed the morphology of synthesized nanostructures. fig. 4 (a) shows plate-like structures of pristine ZnO. The alteration in morphology is observed after addition of amino acids. In case of amino acid assisted ZnONSs, the rod like and flowery structures have been obtained as in fig. 4 (b-i).

The elemental analysis was performed using EDS Spectroscopy attached to SEM to assess the elemental composition of prepared nanostructures. ED's spectra are shown in Fig. 5 for corresponding pristine and amino acid assisted ZnONSs. It is validated from the results that two elements, i.e., Zn and O, are present as main elements in the pristine and amino acid assisted ZnONSs.

Evaluation of antimicrobial potential of nanostructures was done against clinically isolated bacteria i.e. E. coli MBL-E24 and S. aureus MBL-S16. The aforementioned bacteria were obtained from the Molecular Biology Laboratory, LUMHS, Jamshoro, and grown in nutrient broth. The antibacterial assay was carried out using the disc diffusion method against two bacterial species, i.e., E. coli and S. Aureus. Ampicillin was used as reference and pristine ZnO was used as negative control. The results obtained from antibacterial effects were compared with those of synthesized ZnONSs. The zone of inhibition is depicted in Fig. 6 (a-b) while Fig. 7 (a-b) shows the mean results with standard deviation of antibacterial response of both pristine and amino acid assisted ZnONSs. The comparative analysis is separately given in table 2. The reference values of E. coli and S. Aureus are mentioned in table 3. Both types of samples exhibit antibacterial activity against Gram-positive and Gram-negative bacteria. The results suggest that ampicillin was found to be intermediately resistant towards E. Coli with area of inhibition of 7.8 mm.

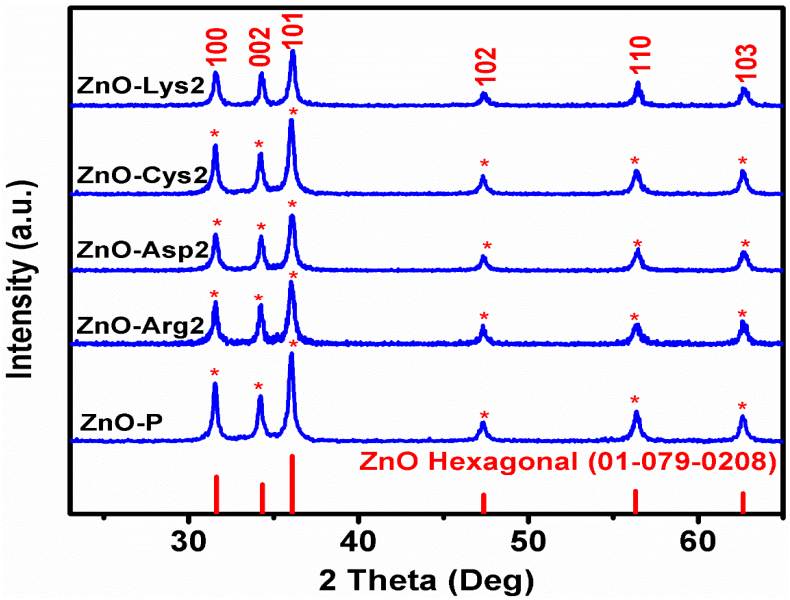

Fig. 1: XRD patterns of pristine ZnONS and various amino acid assisted ZnONSs

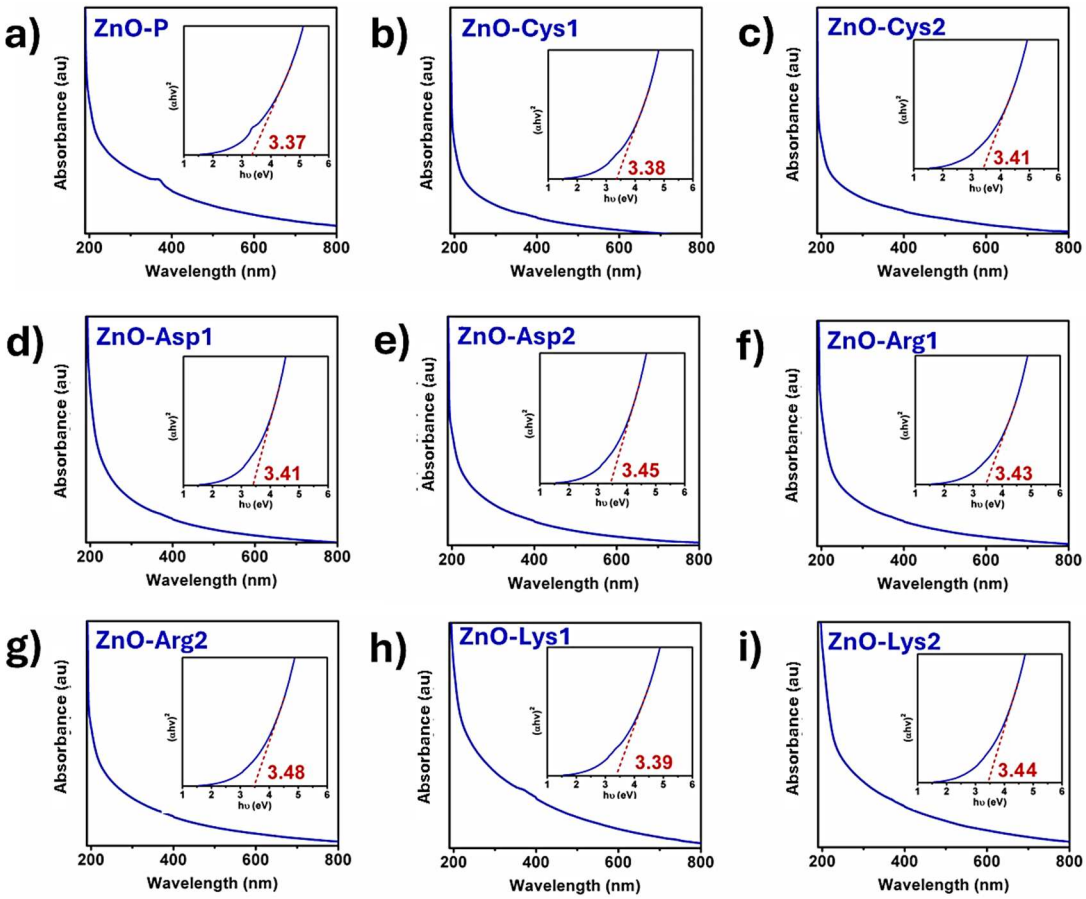

Fig. 2: UV-Vis absorption spectra of various synthesized samples and corresponding energy band gap calculated using Tauc Plot (a) ZnO-P, (b) ZnO-Arg1, (c) ZnO-Arg2, (d) ZnO-Asp1, (e) ZnO-Asp2, (f) ZnO-Cys1, (g) ZnO-Cys2, (h) ZnO-Lys1, and (i) ZnO-Lys2

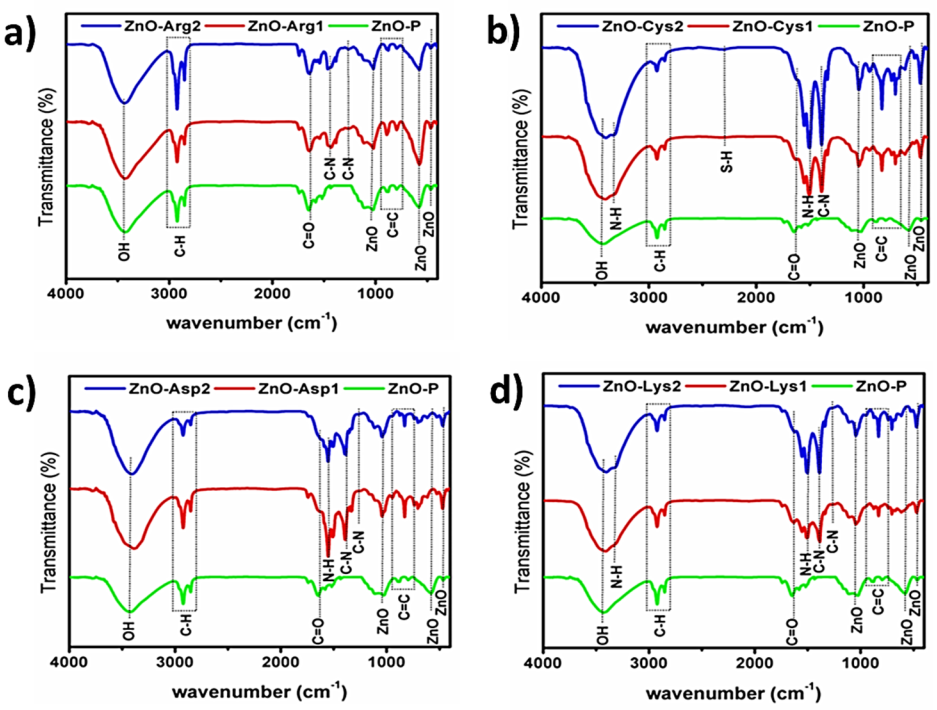

Fig. 3: FTIR spectra of different amino acid-based samples accompanied with pristine ZnO (a) Arginine, (b) Cystine, (c) Aspartic Acid and (d) Lysine

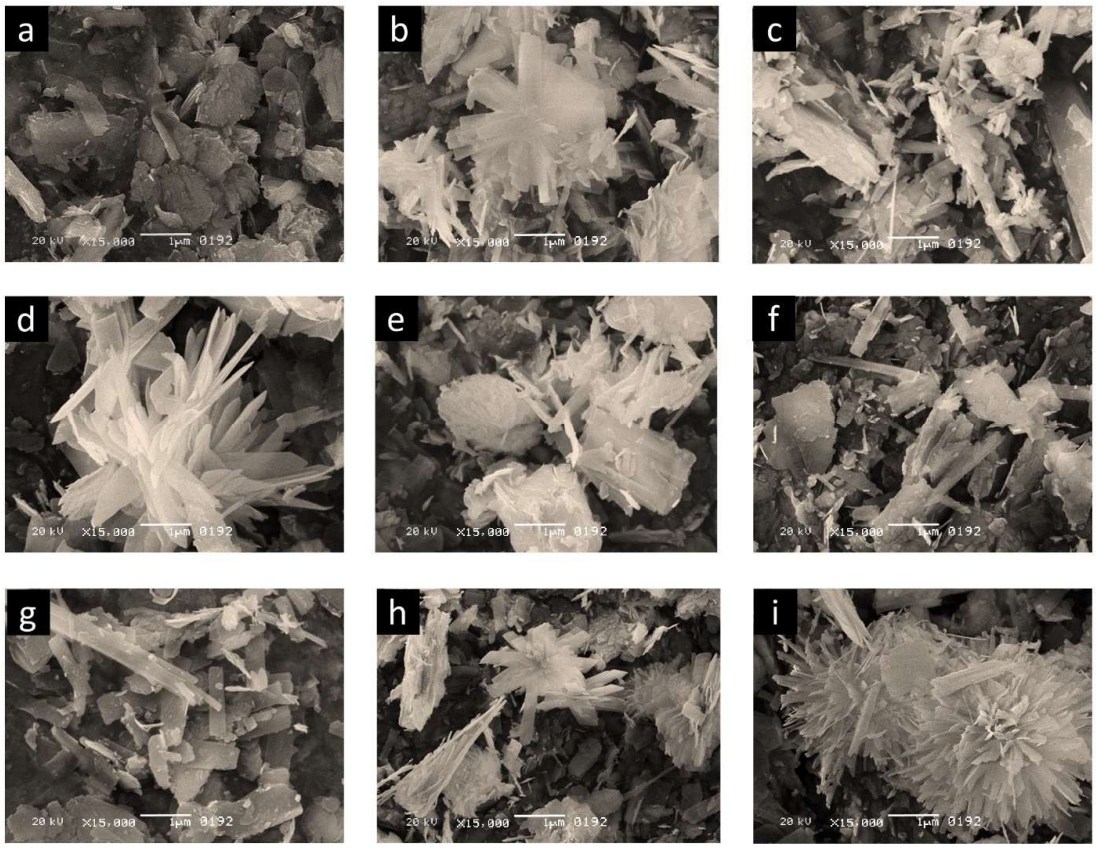

Fig. 4: SEM images of prepared samples (a) ZnO-P, (b) ZnO-Arg1, (c) ZnO-Arg2, (d) ZnO-Asp1, (e) ZnO-Asp2, (f) ZnO-Cys1, (g) ZnO-Cys2, (h) ZnO-Lys1, and (i) ZnO-Lys2

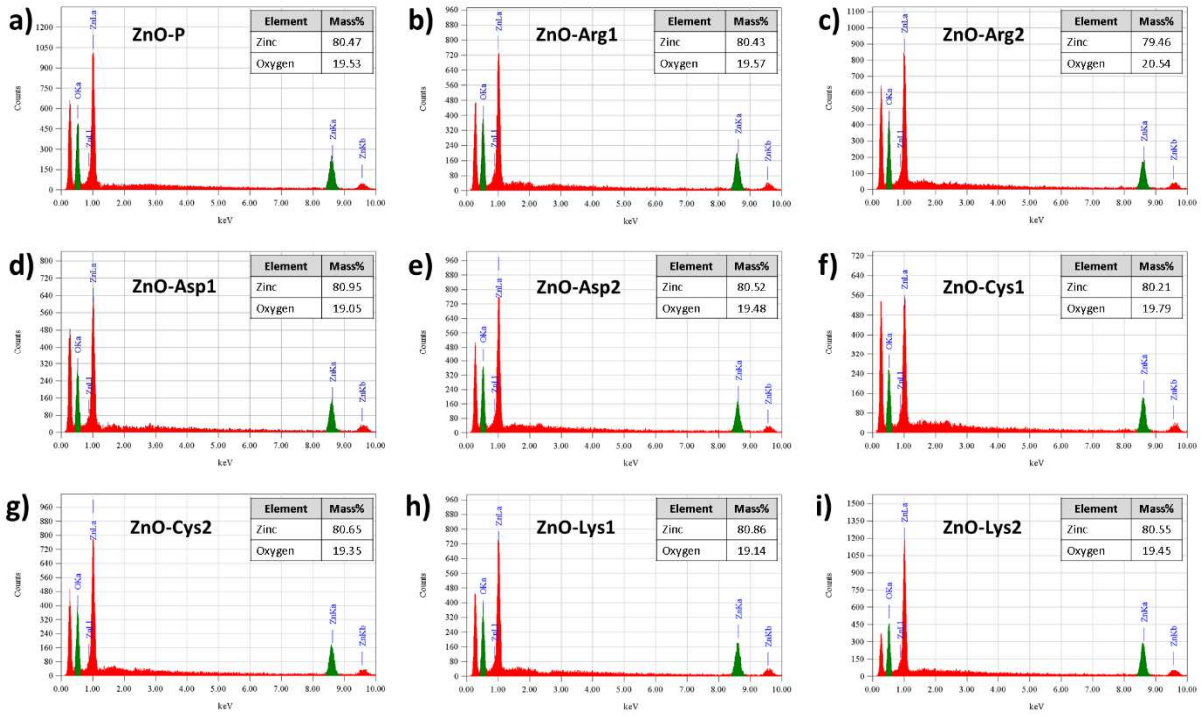

Fig. 5: EDS spectra of (a) Pristine ZnO and (b to i) Amino acid assisted ZnO

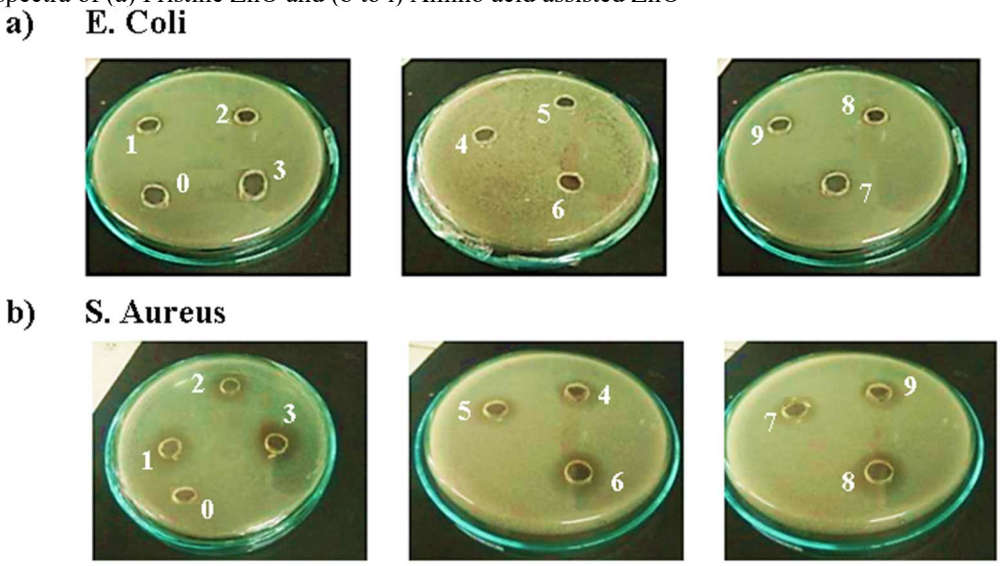

Fig. 6: Antibacterial activity using disc diffusion assay for (a) E. coli and (b) S. Areus

Table 1: The composition of synthesized samples

S. No.	Sample I.D.	Zinc acetate dihydrate	Urea	Type of amino acis	Concentration (Amino acids)
1	ZnO-P	0.1 M	0.1M	Zinc oxide Pristine	Nil
2	ZnO-Arg1	0.1 M	0.1M	Arginine	1 mM
3	ZnO-Arg2	0.1 M	0.1M	Arginine	2 mM
4	ZnO-Asp1	0.1 M	0.1M	Asp. Acid	1 mM
5	ZnO-Asp2	0.1 M	0.1M	Asp. Acid	2 mM
6	ZnO-Cys1	0.1 M	0.1M	Cystine	1 mM
7	ZnO-Cys2	0.1 M	0.1M	Cystine	2 mM
8	ZnO-Lys1	0.1 M	0.1M	Lysine	1 mM
9	ZnO-Lys2	0.1 M	0.1M	Lysine	2 mM

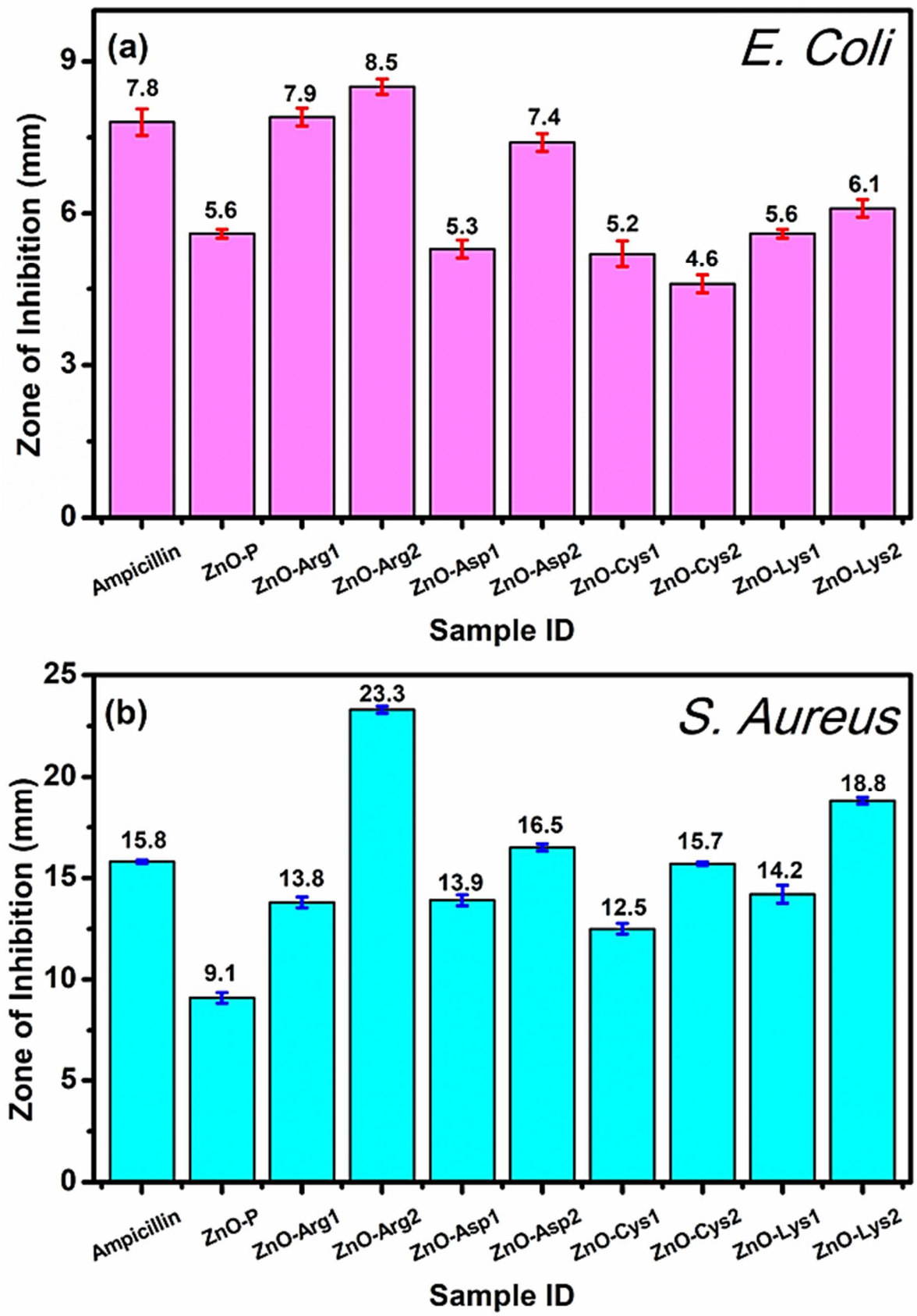

Fig. 7: Comparative antimicrobial activity of amino acid assisted ZnO nanostructures against (a) E. coli and (b) S. Areus

Table 2: Comparative analysis for antibacterial activity of Amino Acid assisted ZnO nanostructures against E. coli and S. Areus

Sample	Microorganisms			
	E. coli		S. Aureus	
	Mean Inhibitor Zone diameter (mm)	Result	Mean Inhibitor Zone diameter (mm)	Result
Ampicillin	7.8	I	15.8	S
ZnO-P	5.6	R	9.1	R
ZnO-Arg1	7.8	I	13.8	I
ZnO-Arg2	8.5	I	23.3	S
ZnO-Asp1	5.3	R	13.9	I
ZnO-Asp2	7.4	I	16.5	S
ZnO-Cys1	5.2	R	12.5	I
ZnO-Cys2	4.6	R	15.7	S
ZnO-Lys1	5.6	R	14.2	I
ZnO-Lys2	6.1	R	18.8	S

R = Resistant; I = Intermediate resistance; S = Sensitive

Table 3: Zone diameter interpreter chart

Microorganism	Resistant (R)	Intermediate resistance (I)	Sensitive (S)
	Diameter (mm)	Diameter (mm)	Diameter (mm)
E. coli	<7	7-10	>10
S. Areus	<10	10-15	>15

Pristine ZnO was used as a negative control, and its antibacterial activity was found to be resistant to E. coli, with an area of inhibition of 5.6 mm. In the case of amino acid assisted ZnONSs samples, the results obtained from antibacterial studies were different, ranging from intermediate resistance to resistance for E. coli. Wherein, ZnO-Arg1, ZnO-Arg2 and ZnO-Asp2 were intermediate resistant against E. coli, showing their inhibition areas 7.8, 8.5 and 7.4 mm, respectively, whereas ZnO-Asp1, ZnO-Cys1, ZnO-Cys2, ZnO-Lys1 and ZnO-Lys2 were found to be resistant against E. coli, containing different zones of inhibition, including 5.3, 5.2, 4.6, 5.6 and 6.1 mm, respectively.

Furthermore, ampicillin was found to be sensitive against S. aureus, with an area of inhibition of 15.8 mm. The antibacterial effects of pristine ZnO were found to be resistant towards *S. Aureus* with an area of inhibition of 9.1 mm. Similarly, in amino acid assisted ZnONSs samples, the results obtained from antibacterial studies were different, ranging from intermediate resistance to resistance against *S. Aureus*. In case of ZnO-Arg1 (13.8 mm), ZnO-Asp1 (13.9 mm), ZnO-Cys1 (12.5 mm) and ZnO-Lys1 (14.2 mm) were intermediate resistant against *S. Aureus.* While ZnO-Arg2 (23.3 mm), ZnO-Asp2 (16.5 mm), ZnO-Cys2 (15.7 mm) and ZnO-Lys2 (18.8 mm) zones found to be sensitive against *S. Aureus* having different zones of inhibitions. Among these amino assisted ZnONSs, ZnO-Arg2 exhibit superior antibacterial activity for *S. Aureus* as compared to counter peers.

DISSCUSSION

The XRD patterns were compared with those from the previously published papers on ZnONSs. From results, it was confirmed that due to addition of amino acids, diffraction peaks were found to be weak in XRD patterns of amino acid assisted ZnONSs, as shown in Fig. 1. Due to lattice distortion, low peak intensity was observed in XRD pattern, which is an indication of a low degree of crystallinity (Mou Pal, 2012, Terohid, 2018).. The data validates that no impurity was present in XRD analysis. UV Visible patterns were compared with already published paper. A strong absorption peak in the range of 350-365 nm was observed, which is the characteristic peak of ZnO as reported in earlier studies (Pudukudy, 2014, Singh, 2012). The nanostructures synthesized in the presence of amino acid do not show any absorption peak within the range of 350-365 nm, indicating a significant alteration in the optical response of ZnO due to amino-acid-assisted synthesis. Tauc plot is given inside the corresponding figures with hυ along the x-axis while $(\alpha h\upsilon)^2$ on the Y axis, extrapolating the curve to meet X axis. i.e. $(\alpha h\upsilon)^2= 0$. This value on the x-axis corresponds to optical band gap energy. Fig. 2(a) shows the energy band gap of 3.37 eV for pristine ZnO. In range of 3.30 eV to 3.50 eV, band gap energy verifies the presence of ZnONSs. While Amino acid assisted ZnONSs contain 3.38, 3.41, 3.41, 3.45, 3.43, 3.48, 3.49, and 3.44 eV energy band gaps for ZnO-Cys1, ZnO-Cys2, ZnO-Asp1, ZnO-Asp2, ZnO-Arg1, ZnO-Arg2, ZnO-Lys1 and ZnO-Lys2, respectively, shown in fig. 2 (b to i). An increase in band gap energy was revealed after the addition of amino acids, which is due to the quantum confinement effect (Raji, 2017; Saxena, 2022; Singh, 2023; Suresh, 2017). Additionally, it is observed that ZnO-Arg2 represents the highest energy band gap compared to others. The results of both XRD and UV-Vis agreed with each other.

The FTIR is a technique used for analysis/confirmation of functional groups of a compound or nowadays most

frequently used for confirmation of nanostructures. The ZnONSs were prepared and their functional group was confirmed using this technique. The C=O band peak exhibits at 1640 cm^{-1} as reported in literature (El-Khawaga, 2023, Mohd Yusof, 2020, Selvaraj, 2019). These above peaks are common for all samples due to presence of ZnONS. However, in amino acid assisted ZnONSs, they contain extra peaks, i.e., 1385 and 1230 cm-1, that are attributed to the C-N bond, which is present in all amino acid samples, such as Arginine, Lysine, L-cystine, and Aspartic acid. Furthermore, L-cystine, Lysine, and Aspartic acid assisted sample also contains N-H bond at 3310 cm−1 and 1495 cm−1, which shows the presence of an ammonium group. Furthermore, Cystine represents the vibration band at 2294 cm^{-1} due to the presence of the S-H bond for the thiol group (Alexander, 2016; Devi, 2016; Dhal, 2022; Kogelheide, 2016; Selvaraj, 2019). These FTIR findings also suggest that amino acids are present in synthesized ZnONSs.

The SEM is also utilized to check morphology of synthesized nanostructures. The only thing that was observed in newly synthesized nanostructures was an increase in surface area. It was also witnessed after addition of amino acids, this modification of morphology can increase their antibacterial activity because of this reason.

The EDS data was also analyzed and validated. The analysis of all samples revealed that the weight percentage of Zn was approximately four times higher than that of O, confirming the formation of the ZnO phase in the synthesized nanostructures. These findings are in strong agreement with the XRD and UV–Visible analyses, confirming the successful synthesis of pristine ZnO nanostructures (ZnONSs) as well as amino-acid-assisted ZnONSs. The antibacterial assay of Ampicillin, pristine ZnO and amino acid assisted ZnONSs were carried out using disc diffusion method against two bacterial species i.e. E. coli and S. Aureus. Ampicillin was used as reference and pristine ZnO was used as negative control. The antibacterial performance of the synthesized ZnONSs was assessed through comparative analysis of the inhibition zones. Among all samples, arginine-assisted ZnONSs exhibited the most superiour antibacterial activity against both bacterial species. This enhanced antibacterial efficacy is attributed to the presence of the positively charged guanidinium group of arginine which enhances electrostatic interactions with negatively charged bacterial membranes, improving nanoparticle adhesion and facilitating membrane disruption. The reason if positively charged guanidinium group of arginine enhances electrostatic interactions with negatively charged bacterial membranes, improving nanoparticle adhesion and facilitating membrane disruption. Arginine functionalization also tailors the morphology of ZnONSs toward rod-like and flower-like architectures, which provide higher surface area and defect density, thereby amplifying reactive oxygen species (ROS) generation and antibacterial potency. Arginine-assisted ZnO (Arg-2) nanostructures exhibit superior antibacterial activity due to the synergistic effects of ZnO's intrinsic mechanisms, arginine's guanidinium chemistry and distinctive morphology. Furthermore, arginine may contribute to nitric oxide (NO)-related pathways, intensifying oxidative stress in bacterial cells. In addition, arginine capping regulates Zn^{2+} ion release, supporting sustained bactericidal effects. Therefore, the combined chemical interactions and morphology-related features account for the superior antibacterial performance of arginine-assisted ZnONSs, with ZnO-Arg2 in particular exhibiting significantly larger inhibition zones compared to other amino acid-assisted counterparts (Abou Zeid et al., 2023, (Araiza-Campos et al., 2023) (Kang et al., 2024). The other nanostructures were either less or very much less potent than arginine assisted ZnONSs.

CONCLUSION

A simple green synthesis route was employed for the preparation of ZnO nanostructures (ZnONSs) using four amino acids-arginine, aspartic acid, cystine and lysine-as stabilizing agents. The synthesized ZnONSs were characterized using SEM, XRD, UV–Vis, FTIR and EDS techniques. Morphological analysis revealed rod-like and flower-like structures with enhanced surface area upon amino acid addition. EDS confirmed the dominance of Zn in elemental composition, while XRD patterns displayed weaker diffraction peaks, indicating reduced crystallinity. FTIR spectra validated the presence of amino acid–associated functional groups on the ZnONS surface. Antibacterial activity, assessed by the disc diffusion method, demonstrated that the zone of inhibition varied depending on the amino acid used. Notably, ZnO–Arg1 and ZnO–Arg2, as well as ZnO–Asp2, exhibited larger inhibition zones against E. coli compared to ampicillin. Against *S. aureus*, ZnO–Arg2, ZnO–Asp2 and ZnO–Lys2 showed superior inhibition compared to ampicillin. Among all, ZnO–Arg2 nanostructures displayed the most potent antibacterial activity, highlighting the role of amino acid selection in tailoring antimicrobial performance. The superior activity of arginine-assisted ZnONSs may be attributed to the presence of positively charged guanidinium groups, which enhance electrostatic interactions with negatively charged bacterial membranes.

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Authors' contributions

Sara Aftab: Prepared the nanostructured material and wrote the concerned part of the manuscript; Ibtessam Tahir

Ansari: Overall supervised the work and wrote the relevant part of the manuscript; Umair Aftab: Performed SEM/ EDS and analyzed it; Rustem Zairov: Analyzed physical characterization, including XRD, UV-vis spectroscopy and FTIR; Mohsin Ali: Analyzed the antibacterial activity of the nanostructured materials; Saeed Ahmed Lakho: Wrote the manuscript part with some characterizations, such as UV-Vis, FTIR, and XRD, and finalized the manuscript.

Ethical approval

This study did not involve human participants or experimental animals. All antibacterial assays were conducted in vitro using standard laboratory bacterial strains and followed established biosafety and ethical guidelines. No ethical approval was required for this research.

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Data availability statement

The data is available with corresponding authors and can be shared on request.

Conflicts of interest

The authors of this paper have acknowledged no conflicts of interest

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