Global **A**dvances in **M**ultidisciplinary **A**pplications in **N**ext-Gen **A**nd **M**odern Technologies (**GAMANAM)**
Volume 1, Issue 1, January 2025, Pp: 45-51

Research Paper

Green Synthesis of Chitosan-Functionalized Zinc Oxide Nanoparticles - A Novel Antimicrobial Agent

[1]Harika Katepogu, [2*]P Josthna, [2]M Shakari, [2]Dara Josphin, [2]P.Bharathi, [1]Shilpa Nayuni

[1]Arran Scientific, Madanapalle, Annamayya Dt, Andhra Pradesh, Indian.
[2]Department of Biotechnology, Sri Padmavati Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, India.

**Corresponding Author(s): penchalajyo@yahoo.co.in*

Received: 13/10/2024, Revised: 25/11/2024, Accepted:28/12/2024 Published:12/01/2025

Abstract: This study presents a green synthesis method for Chitosan-functionalized zinc oxide (ZnO) nanoparticles using guava (Psidium guajava) leaf extract and Chitosan, a natural polymer derived from chitin. The eco-friendly approach leverages guava extract as a reducing and stabilizing agent and Chitosan to enhance nanoparticle stability and bioactivity. Synthesized ZnO nanoparticles exhibited a crystalline structure with sizes ranging from 14–28 nm, confirmed by XRD analysis, and a characteristic UV-Visible absorption peak at 335 nm. The antimicrobial activity of the nanoparticles was evaluated against E. coli, S. aureus, and S. enterica using a disc diffusion assay. Significant inhibition zones were observed, particularly at higher nanoparticle concentrations, indicating strong antibacterial potential. The mechanism involves oxidative stress induction and bacterial membrane disruption. This green synthesis approach provides an effective and sustainable alternative to combat antimicrobial resistance, aligning with green chemistry principles to minimize environmental impact.

Keywords: Green synthesis, Chitosan-functionalized ZnO nanoparticles, Antimicrobial activity, Guava extract

1. Introduction

According to the Merriam-Webster Medical Dictionary, an antimicrobial is an agent that prevents or stops the growth of pathogenic microorganisms or even kills them [Kingston, *et al.,2008*]. Antimicrobial agents have been used for at least 2000 years, originating in the time of the Ancient Egyptians. These agents play a critical role in treating infectious diseases and are essential in modern medicine. They can be classified based on their target organisms, chemical structure, mode of action, or source of origin. To date, many types of antimicrobials have been developed, classified based on their target organisms and mode of action. Examples based on target organisms include: antibacterial agents (targets bacteria) such as penicillin, tetracycline, antivirals (target viruses) such as acyclovir, oseltamivir, antifungals (target fungi) such as fluconazole, amphotericin B, antiparasitics (target parasites) such as ivermectin, chloroquine. Examples based on mode of action include bactericidal agents (kill bacteria) such as beta-lactams, aminoglycosides, bacteriostatic agents (inhibit bacterial growth) such as tetracyclines, sulfonamides. Antimicrobial agents typically work by inhibiting cell wall synthesis, nucleic acid synthesis, or metabolic pathways. In recent times, antimicrobial resistance has become a major global health threat, primarily caused by the overuse or misuse of antimicrobials. Ideal antimicrobial agents should target microorganisms without harming the host, while factors such as absorption, distribution, metabolism, and excretion influence their efficacy. A modern approach to combating antimicrobial resistance would involve finding new targets or using alternative antimicrobials, either natural or synthetic compounds [Mantravadi, *et al*., 2019].

Nanoparticles are a new generation of antimicrobial agents that are being explored. One of the established relationships between nanomaterials and antibacterial activity is as follows: “Nanomaterials as antibacterial complements to antibiotics are highly promising and gaining significant interest as they may address the limitations where antibiotics often fail” [*Mohanpuria et al.,2007*]. Additionally, nanomaterials can complement and support traditional antibiotics “as effective carriers” [*Sunagawa et al., 2004*]. This section focuses on the unique features and complementary advantages of using nanomaterials in antimicrobial applications.

Green synthesis of Nanoparticles aims to promote innovative chemical technologies that reduce or eliminate the use and production of hazardous substances in the design, manufacture, and application of chemical products. This approach focuses on minimizing, or ideally, eliminating, pollution produced during synthesis, avoiding the consumption and waste of non-renewable raw materials,

using safer alternatives in product manufacturing, and reducing synthesis time (*Paul Anastas, et al.,2004*).

Guava (*Psidium guajava)* is a Phyto therapeutic plant used in folk medicine, believed to contain active components that help treat and manage various diseases. Many parts of the plant have been used in traditional medicine to manage conditions such as malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothaches, coughs, sore throat, inflamed gums, and several other conditions [Jaiarj and Khoohaswan., *et al., 1999*]. This plant has also been used to control life-changing conditions like diabetes, hypertension, and obesity [South-East Asian *et al., 2006*]. Guava is increasingly being explored as a natural source for the green synthesis of nanoparticles (NPs). Extracts from guava leaves, fruits, and bark contain phytochemicals such as flavonoids, tannins, and polyphenols, which act as reducing and stabilizing agents in nanoparticle synthesis.

Chitosan, a natural antimicrobial agent found in the shells of crustaceans, such as crabs, shrimp, squid pens, and crawfish (No *et al. 2002*). Recently, some studies have suggested the possibility of producing Chitosan from fungi. In one study, Chitosan was extracted from the cell wall of filamentous fungus, *R. oryzae*, (Jeihanipour *et al., 2007*) and its antimicrobial properties were studied against *E. coli, K. pneumoniae and S. aureus* (Hosseinnejad and Jafari, *2016)*. Guava (*Psidium guajava*) and Chitosan, a natural polymer derived from chitin (found in crustacean shells), offer complementary properties that make them ideal candidates for applications in nanotechnology. Both materials are biocompatible, biodegradable, and rich in bioactive compounds, enabling their combined use in the synthesis and functionalization of nanoparticles (NPs) for antimicrobial, pharmaceutical, and environmental applications

ZnO nanoparticles are believed to be among the three most produced nanomaterials, alongside titanium dioxide nanoparticles and silicon dioxide nanoparticles (Zhang, Yuanyuan, *et al., 2015*). The most common use of ZnO nanoparticles is in sunscreen. ZnO nanoparticles have been shown to exhibit properties such as anti-cancer, antidiabetic, antibacterial, antifungal, anti-inflammatory activities.

Considering the activities and benefits of guava, Chitosan, and zinc nanoparticles, this work will explore the green synthesis of Chitosan-functionalized zinc oxide nanoparticles as a novel antimicrobial agent.

2. Materials and Methods

Materials

Zinc acetate (0.1983g), Guava plant extract (22g), NaOH, Chitosan (1g), Acetic acid (10ml) NaoH, 1% Citric acid, Agar broth, Antibiotic disc, Eppendorf tubes, Inoculums, Forceps, Metric ruler, Bunsen burner, Cotton swab, Pipettes, Glass rod.

Methods

Collection of Plants

Fresh leaves of *Psidium guajava* (guava) were collected from the open areas of Arran Science Laboratory, Valasapalli, Chittoor district. The collected leaves were washed with running tap water, followed by distilled water, and then dried at room temperature.

Preparation of plant extract

The plant extract was prepared by grinding 22g of leaves into a paste using an electric mixer. A leaf broth solution was prepared by adding 220ml of distilled water to the 22g of plant paste in a 1:10 ratio. The mixture was then filtered through standard filter paper, with Whatman No. 1 filter paper. The remaining extract was stored at 4°C for further experiments.

Figure 1: Guava extract

Preparation of stock solution

To 500 ml of distilled water, 10 ml (0.9183g) of zinc acetate was added. The mixture was stirred using a magnetic stirrer to form a stock solution.

Biosynthesis of zinc oxide nanoparticles (ZnO NPs):

To 50 ml of the prepared zinc acetate solution, 10 ml of the plant extract was added dropwise until the solution becomes colourless or turned white. NaOH was then added to bring the pH to 10. The solution was placed on a hot plate to form powder.

Figure 2: Zinc acetate

Preparation of Chitosan:

A 1g of chitosan was mixed in 100ml of distilled water. Since distilled water cannot dissolve Chitosan, acetic acid was added drop by drop until a clear solution was obtained.

Then, 30ml of Chitosan solution was mixed with 30ml of plant extract with zinc acetate. The solution was stirred for 24 hours with a magnetic stirrer. After that, it was autoclaved for 15 minutes at 121^0C. Once cooled, the solution was stored in the refrigerator for 24 hours. To allow the solution to form a powder, it was transferred into a Petri dish and placed on the hot plate at 70^0C until the liquid evaporated.

Figure 3: Chitosan

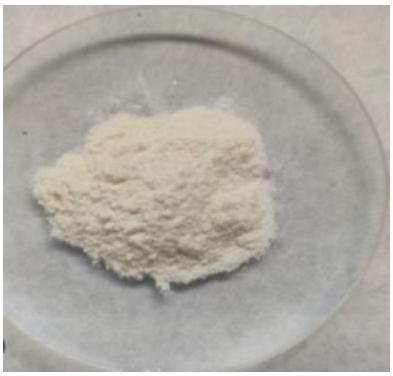

Figure 4: Chitosan Powder

Preparation of Guava-Chitosan stabilized zinc nanoparticle solution for antimicrobial activity:

A 1% citric acid solution (20 mL) was added to 30 mL of the prepared Guava-Chitosan stabilized zinc nanoparticle solution. Serial dilutions were then prepared by taking 25 µL, 50 µL, 75 µL, and 100 µL of the nanoparticle solution, respectively, and diluting them accordingly.

Figure 5: Guava- Chitosan stabilized Zinc nanoparticles with citric acid solution

Table 1: Chitosan dilution concentrations

S.No	Chitosan Solution	Water
1	100µl	-
2	75µl	25µl
3	50µl	50µl
4	25µl	75µl

Disc diffusion assay:

The working area was sterilised with disinfectant, and sterile cotton was soaked in the inoculum. The excess medium was removed, and the inoculum was aspirated by pressing the swab against the wall of the tube. Afterward, the plate was dried for 5 minutes to allow proper inoculation detection. Forceps were sterilized with alcohol before handling the antibiotic discs. The antibiotic discs were placed in the centre of the plate. Holes were made on the four sides of the plate using the tips. Then prepared solution was added slowly, drop by drop, and the plates were incubated upside down for 24 hours at 37°C. After 24 hours of incubation, the zone of inhibition was measured with a scale and the diameter was recorded.

3. Results and Discussion

The synthesis of Chitosan stabilized zinc nanoparticles was observed through a colour change from a colourless solution to a white colour coloured solution as shown in the Fig 6. The green synthesised Chitosan/ZnO nanoparticles transitioned from colourless to a milky solution, as shown in Figure 7. The first attempt at synthesizing ZnO nanoparticles involved reducing zinc nitrate with sodium hydroxide (NaOH) without using a stabilizing agent, leading to agglomeration and a non-homogeneous solution. This can be explained by the addition of zinc nitrate to the NaOH solution, produces a milky precursor solution. This is the initial stage of the zinc nitrate reduction reaction, where zinc nitrate dissociates into Zn^{2+} and NO_3^- ions. After stirring for several minutes, suspensions formed in the solution due to the agglomeration process. Without a stabilizing agent to coat the nanoparticle surfaces, agglomeration began to occur. If the reduction process precedes the interaction with the stabilizing agent, nanoparticle growth cannot be effectively controlled, leading to clusters.

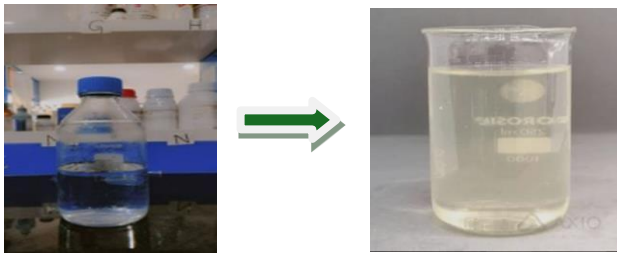

Fig 6: Colour changes from colourless to white colour

Fig 7: Guava plant extract

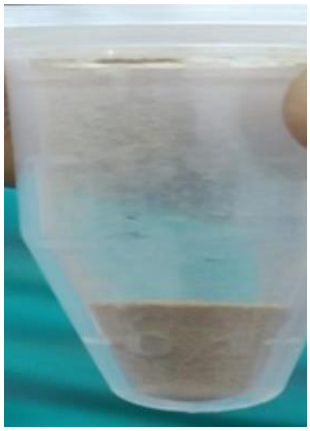

Fig 8: Zn acetate powder

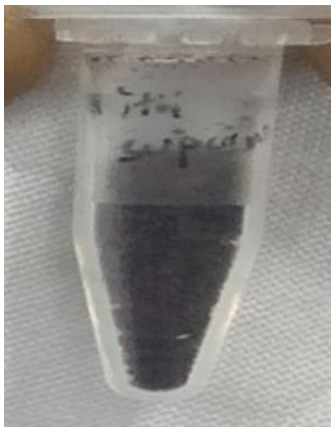

Fig 9: Chitosan stabilized Zn powder

XRD analysis:

XRD analysis of Zinc with Chitosan:

The XRD pattern of Chitosan-stabilised ZnO showed intense peaks at 2θ values of 28.4304, 29.1562, 30.9185, 41.5382. The particle size of ZnO with Chitosan is 28.4304nm.

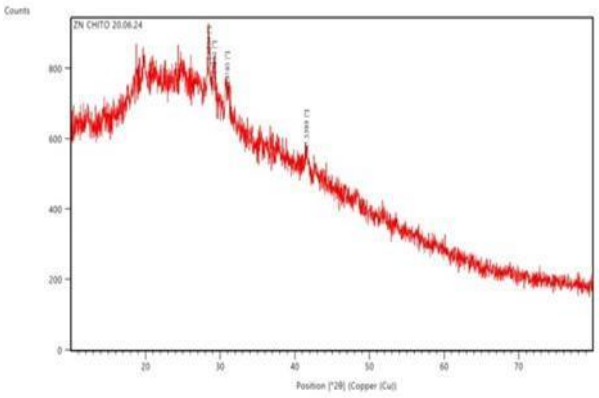

Fig:10: XRD pattern of zinc with Chitosan

XRD analysis of Zinc NPS with *Psidium guajava*:

The XRD pattern of ZnO NPs synthesized using ***Psidium guajava*** extract showed intense peaks at 2θ values of 14.2576, 28.4202 and 31.2820 The particle size of ZnO NPs synthesized by *Psidium guajava* is 14.2576nm.

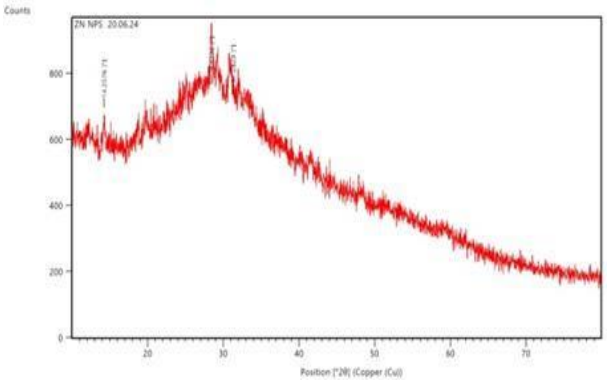

Fig 11: XRD pattern of zinc NPs by *Psidium guajava*

XRD analysis of Stabilized ZnO with *Psidium guajava*:

The XRD pattern of ZnO NPs synthesized using ***Psidium guajava*** extract showed intense peaks at 2θ values of 14.3469, 18.6823, 19.8046, 24.9359, 26.7010, 29.1592, 30.9674. The particle size of stabilized ZnO NPs by *Psidium guajava* is 14.3469nm.

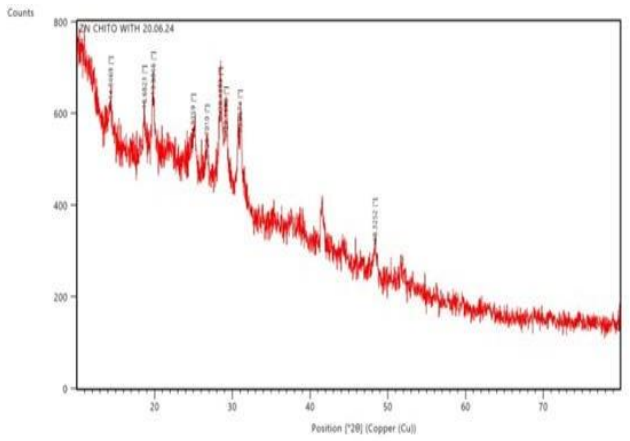

Fig 12: XRD pattern of stabilized ZnO NPs by *Psidium guajava*

The analysis aimed to determine the size of various crystalline forms of nanoparticles. The XRD patterns confirmed that the Chitosan/ZnO nanoparticles exhibited a well-defined crystalline structure. Comparison of the XRD spectra with the standard peaks from the JCPDS files showed excellent matching for all samples. No impurities were observed in the characteristic peaks, indicating the purity of the synthesized nanoparticles. The choice of Chitosan as the stabilizing polymer was validated, as it effectively prevented aggregation and sedimentation. The average crystallite size of the Chitosan/ZnO nanoparticles calculated using the Debye Scherrer formula.

UV-Visible spectroscopy:

The UV-Visible spectrum analysis of Chitosan-stabilized ZnO nanoparticles showed distinct absorption peaks, indicative of their unique optical properties. A prominent absorption peak was observed at 335 nm, characteristic of ZnO nanoparticles. This peak indicates the presence of ZnO in its nanoparticulate form and corresponds to the band gap transition. The position and intensity of the absorption peak suggest that the size and optical behaviour of the nanoparticles are influenced by the stabilizing effect of Chitosan**.**

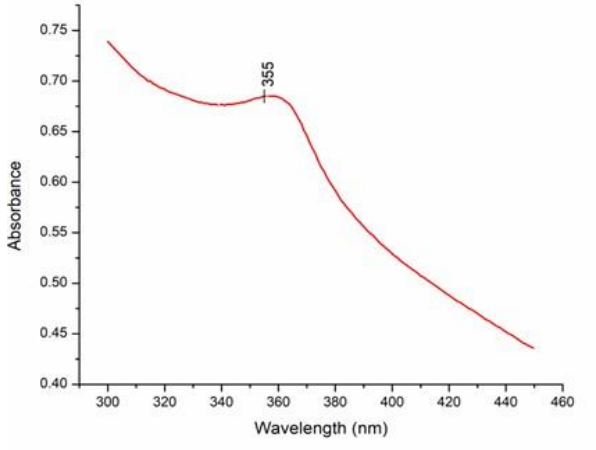

Figure 13: UV-visible Spectra of synthesized nanoparticles

Anti-microbial activity:

The anti-microbial activity of Chitosan-stabilized ZnO nanoparticles was evaluated against three bacterial strains: *E. coli*, *S. aureus*, and *S. enterica* as shown in table 2. The zones of inhibition were observed to increase with higher concentrations of nanoparticles, showing a dose-dependent response. At the highest concentration (100 µL), the largest zones of inhibition were recorded for *S. aureus* (21 mm) and *S. enterica* (23 mm), indicating strong antibacterial effects. *E. coli* also showed a significant inhibition zone of 21 mm at the same concentration. These results confirmed that Chitosan-stabilized ZnO nanoparticles were effective against both Gram-positive and Gram-negative bacteria. In comparison, the control group with a standard antibiotic showed consistent inhibition zones of 20 mm across all bacterial strains, suggesting similar antimicrobial activity between the nanoparticles and the antibiotic. At lower nanoparticle concentrations, *S. aureus* displayed the smallest zones of inhibition, while *S. enterica* showed a more prominent increase in inhibition as the nanoparticle concentration rose. Overall, the findings support the potential of Chitosan-stabilized ZnO nanoparticles as effective antibacterial agents, with higher concentrations providing stronger antimicrobial effects.

Table 2: Antimicrobial activity of different concentrations of green synthesised Chitosan-stabilized ZnO nanoparticles against three bacterial strains: *E. coli*, *S. aureus*, and *S. enterica*.

S.No	Concentration (µl)	Zone of inhibition (*E. coli*) (mm)	Zone of inhibition (*S. aureus*) (mm)	Zone of inhibition (*S. enterica*) (mm)
1	25	17	5	14
2	50	18	13	17
3	75	20	17	19
4	100	21	21	23
5	Control	20	20	20

Fig 14: Antimicrobial activity of silver nanoparticles against *E. coli*, *S. aureus* and *S. enterica*

4. Conclusion

Zinc oxide (ZnO) nanoparticles were synthesized through a green synthesis method, using zinc acetate dihydrate and an extract from guava leaves. The resulting nanoparticles had an average crystallite size of 14.13 nm.

The antibacterial activity of the Chitosan-stabilized ZnO nanoparticles was tested in vitro, showing notable effectiveness against both Gram-positive and Gram-negative bacterial strains. The observed antibacterial action is likely due to the nanoparticles ability to generate reactive oxygen species (ROS), induce oxidative stress, and physically damage bacterial cell membranes, ultimately leading to bacterial inhibition or cell death.

References

[1] Abdelrahim, S. I., Almagboul, A. Z., Omer, M. E. A., & Elegami, A. (2002). Antimicrobial activity of *Psidium guajava L*. *Fitoterapia*, *73*(7-8), 713-715.

[2] Abdel-Mawgoud, A. M. R., Tantawy, A. S., El-Nemr, M. A., & Sassine, Y. N. (2010). Growth and yield responses of strawberry plants to chitosan application. *European Journal of Scientific Research*, *39*(1), 170-177.

[3] Anastas, P. T., & Warner, J. C. (2000). *Green chemistry: theory and practice*. Oxford university press.

[4] Arumugam, A., Karthikeyan, C., Hameed, A. S. H., Gopinath, K., Gowri, S., & Karthika, V. (2015). Synthesis of cerium oxide nanoparticles using Gloriosa superba L. leaf extract and their structural, optical and antibacterial properties. *Materials Science and Engineering: C*, *49*, 408-415.

[5] Beveridge, T. J. (1999). Structures of gram-negative cell walls and their derived membrane vesicles. *Journal of bacteriology*, *181*(16), 4725-4733.

[6] Burkill H. M.,(1997) The Useful Plants of West Tropical Africa, 2nd edition

[7] Chen, R. H., Domard, A., Muzzarelli, R. A., Tokura, S., & Wang, D. M. (2011). Advances in chitin/chitosan science and their applications. *Carbohydrate polymers*, *2*(84), 695.

[8] Daniel, M. C., & Astruc, D. (2004). Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chemical reviews*, *104*(1), 293-346.

[9] Dhillon, G. S., Brar, S. K., Kaur, S., & Verma, M. (2012). Green approach for nanoparticle biosynthesis by fungi: current trends and applications. *Critical reviews in biotechnology*, *32*(1), 49-73.

[10] Durand, G. A., Raoult, D., & Dubourg, G. (2019). Antibiotic discovery: history, methods and perspectives. *International journal of antimicrobial agents*, *53*(4), 371-382.

[11] Fleming, A. (1929). On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. influenzae. *British journal of experimental pathology*, *10*(3), 226.

[12] Jaiarj, P., Khoohaswan, P., Wongkrajang, Y., Peungvicha, P., Suriyawong, P., Saraya, M. S., & Ruangsomboon, O. (1999). Anticough and antimicrobial activities of *Psidium guajava* Linn. leaf extract. *Journal of Ethnopharmacology*, *67*(2), 203-212.

[13] Jiang, J., Pi, J., & Cai, J. (2018). The advancing of zinc oxide nanoparticles for biomedical applications. *Bioinorganic chemistry and applications*, *2018*(1), 1062562.

[14] Kaushik N, Thakkar MS, Snehit S, Mhatre MS, Rasesh Y, Parikh MS (2010) Biological synthesis of metallic nanoparticles. NanomedNanotechnolBiol Med 6:257–262

[15] Kingston, W. (2008). Irish contributions to the origins of antibiotics. *Irish journal of medical science*, *177*, 87-92.

[16] Kong, M., Chen, X. G., Xing, K., & Park, H. J. (2010). Antimicrobial properties of chitosan and mode of action: a state of the art review. *International journal of food microbiology*, *144*(1), 51-63.

[17] Korbekandi, H., Iravani, S., & Abbasi, S. (2009). Production of nanoparticles using organisms. *Critical reviews in biotechnology*, *29*(4), 279-306.

[18] Bogunia-Kubik, K., & Sugisaka, M. (2002). From molecular biology to nanotechnology and nanomedicine. *Biosystems*, *65*(2-3), 123-138.

[19] Kumariya, R., Sood, S. K., Rajput, Y. S., Saini, N., & Garsa, A. K. (2015). Increased membrane surface positive charge and altered membrane fluidity leads to cationic antimicrobial peptide resistance in Enterococcus faecalis. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1848*(6), 1367-1375.

[20] Iosub, C. Ș., Olăreț, E., Grumezescu, A. M., Holban, A. M., & Andronescu, E. (2017). Toxicity of nanostructures—a general approach. In *Nanostructures for Novel Therapy* (pp. 793-809). Elsevier.

[21] Lo, W. H., Deng, F. S., Chang, C. J., & Lin, C. H. (2020). Synergistic antifungal activity of chitosan with fluconazole against Candida albicans, Candida tropicalis, and fluconazole-resistant strains. *Molecules*, *25*(21), 5114.

[22] Mantravadi, P. K., Kalesh, K. A., Dobson, R. C., Hudson, A. O., & Parthasarathy, A. (2019). The quest for novel antimicrobial compounds: emerging trends in research, development, and technologies. *Antibiotics*, *8*(1), 8.

[23] Makarov, V. V., Love, A. J., Sinitsyna, O. V., Makarova, S. S., Yaminsky, I. V., Taliansky, M. E., & Kalinina, N. O. (2014). "Green" nanotechnologies: synthesis of metal nanoparticles using plants. *Acta Naturae (англоязычная версия)*, *6*(1 (20)), 35-44.

[24] Mohanpuria, P., Rana, N. K., & Yadav, S. K. (2008). Biosynthesis of nanoparticles: technological concepts and future applications. *Journal of nanoparticle research*, *10*, 507-517.

[25] Mukherjee, P., Ahmad, A., Mandal, D., Senapati, S., Sainkar, S. R., Khan, M. I., ... & Sastry, M. (2001). Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis. *Nano letters*, *1*(10), 515-519.

[26] Ncube, N. S., Afolayan, A. J., & Okoh, A. I. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African journal of biotechnology*, *7*(12).

[27] No, H. K., Kim, S. H., Lee, S. H., Park, N. Y., & Prinyawiwatkul, W. (2006). Stability and antibacterial activity of chitosan solutions affected by storage temperature and time. *Carbohydrate polymers*, *65*(2), 174-178.

[28] Noorian, S. A., Hemmatinejad, N., & Navarro, J. A. (2020). Ligand modified cellulose fabrics as support of zinc oxide nanoparticles for UV protection and antimicrobial activities. *International journal of biological macromolecules*, *154*, 1215-1226.

[29] Pasquina-Lemonche, L., Burns, J., Turner, R. D., Kumar, S., Tank, R., Mullin, N., ... & Hobbs, J. K. (2020). The architecture of the Gram-positive bacterial cell wall. *Nature*, *582*(7811), 294-297.

[30] Peña, A., Sánchez, N. S., & Calahorra, M. (2013). Effects of chitosan on Candida albicans: conditions for its antifungal activity. *BioMed research international*, *2013*(1), 527549.

[31] Raafat, D., Von Bargen, K., Haas, A., & Sahl, H. G. (2008). Insights into the mode of action of chitosan as an antibacterial compound. *Applied and environmental microbiology*, *74*(12), 3764-3773.

[32] Raetz, C. R., Reynolds, C. M., Trent, M. S., & Bishop, R. E. (2007). Lipid A modification systems in gram-negative bacteria. *Annu. Rev. Biochem.*, *76*(1), 295-329.

[33] Richard, I., Thibault, M., De Crescenzo, G., Buschmann, M. D., & Lavertu, M. (2013). Ionization behavior of chitosan and chitosan–DNA polyplexes indicate that chitosan has a similar capability to induce a proton-sponge effect as PEI. *Biomacromolecules*, *14*(6), 1732-1740.

[34] Sarwar, S. B., Khondokar, F., Islam, H., Ullah, M. A., Araf, Y., Sarkar, B., & Rahman, H. (2021). Assessing drug repurposing option for emerging viral diseases: concerns, solutions, and challenges for forthcoming viral battles. *J. adv. biotechnol. exp ther*, *4*, 74-94.

[35] Sato, T., Ishii, T., & Okahata, Y. (2001). In vitro gene delivery mediated by chitosan. Effect of pH, serum, and molecular mass of chitosan on the transfection efficiency. *Biomaterials*, *22*(15), 2075-2080.

[36] Sengupta, S., Chattopadhyay, M. K., & Grossart, H. P. (2013). The multifaceted roles of antibiotics and antibiotic resistance in nature. *Frontiers in microbiology*, *4*, 47.

[37] Singh, J., Dutta, T., Kim, K. H., Rawat, M., Samddar, P., & Kumar, P. (2018). 'Green'synthesis of metals and their oxide nanoparticles: applications for environmental remediation. *Journal of nanobiotechnology*, *16*, 1-24.

[38] South-East Asian (SEA), Regional Workshop on Extraction Technologies for Medicinal and Aromatic Plants, 2006.

[39] Sunagawa, M., Shimada, S., Zhang, Z., Oonishi, A., Nakamura, M., & Kosugi, T. (2004). Plasma insulin concentration was increased by long-term ingestion of guava juice in spontaneous non-insulin-dependent diabetes mellitus (NIDDM) rats. *Journal of Health Science*, *50*(6), 674-678.

[40] Wainwright, M. (2008). Some highlights in the history of fungi in medicine–A personal journey. *Fungal Biology Reviews*, *22*(3-4), 97-102.

[41] Xing, K., Zhu, X., Peng, X., & Qin, S. (2015). Chitosan antimicrobial and eliciting properties for pest control in agriculture: a review. *Agronomy for Sustainable Development*, *35*, 569-588.

[42] Zaharoff, D. A., Rogers, C. J., Hance, K. W., Schlom, J., & Greiner, J. W. (2007). Chitosan solution enhances both humoral and cell-mediated immune responses to subcutaneous vaccination. *Vaccine*, *25*(11), 2085-2094.