WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Evaluating the Antimicrobial Potential of Silver Nanoparticles against Two Prominent Mycobacterium Tuberculosis Strains

Ankita Panda[1], Ujjwal Manna[2], Deokrishna Kumar Choudhary[3*]

[1]Department of Biotechnology, Siksha 'O' Anusandhan University in Bhubaneswar, Odisha, India.
[2]Department of Pharmaceutical Biotechnology, Maulana Abul Kalam Azad University of Technology, West Bengal, India.
[3]Mahavir Cancer Sansthan and Research Centre Phulawarisharif Patna, India.
Email: deokrishnak2000@gmail.com
DOI: https://doi.org/10.55248/gengpi.4.923.52274

ABSTRACT:

Tuberculosis (TB) stands as a prominent global health concern. This study aims to assess the impact of silver nanoparticles (AgNP) on *Mycobacterium tuberculosis*, the causative agent of TB. The efficacy of AgNP was determined using the minimum inhibitory concentration (MIC) through a microplate Alamar blue assay. AgNP synthesis involves chemical methods. The size and shape of AgNP were verified through ultraviolet-visible (UV-Vis) absorption spectroscopy, X-ray diffraction (XRD) spectroscopy, and transmission electron microscopy (TEM). Two strains were examined: *Mycobacterium tuberculosis* H37Rv and *Mycobacterium bovis*. Furthermore, a multidrug-resistant *Mycobacterium tuberculosis* strain was included, along with clinically obtained isolates from *Mycobacterium tuberculosis* H37Rv and *Mycobacterium bovis*. The synthesized nanoparticles appeared tetrahedral in shape with an average size of 45±3 nanometers (nm). Results showed that the synthesized AgNPs effectively inhibited the growth of all strains, including the multidrug-resistant strain. The MIC for the multidrug-resistant strain ranged from 2-12 µg/ml, while for *Mycobacterium tuberculosis* H37Rv and *Mycobacterium bovis*, it ranged from 2-14 µg/ml and 3-30 µg/ml, respectively. This study presents a novel approach to combat tuberculosis, a global threat to humanity, highlighting its innovative potential.

KEYWORDS: Silver; nanoparticles; tuberculosis; Mycobacterium; Alamar blue assay, XRD, SEM.

INTRODUCTION:

The world faces numerous critical challenges (Roy and Ray 2019; Roy and Ray 2020), with environmental issues, water scarcity, and human health concerns standing out (Roy, Debnath, and Chakraborty 2022; Roy et al. 2022). Health-related issues are especially significant (Roy et al. 2022; Song et al. 2022; Su et al. 2022), often linked to microorganisms and their products, such as enzymes (Ghoshal et al. 2022; Vipparla et al. 2022).

Tuberculosis is a major global cause of death, attributed to agents like *Mycobacterium bovis* and *Mycobacterium tuberculosis* (Reddington et al. 2011). These pathogens have a high capacity to infect both humans and animals, as reported by the World Health Organization (WHO), causing millions of deaths annually and impacting millions more. *Mycobacterium bovis* poses a global threat, primarily affecting cattle, but transmission to humans occurs through contaminated milk consumption, emphasizing the importance of milk pasteurization (WHO, 2016).

The prolonged treatment duration, increased infection rates, and drug side effects underscore the need for alternative tuberculosis treatments. Prolonged use of conventional drugs has led to the emergence of multi-drug-resistant strains, a global concern (Ducati et al. 2006). The heightened susceptibility of *Mycobacterium bovis* and *Mycobacterium tuberculosis*, particularly in developing countries, has spurred research into the antagonistic potential of metal-based nanoparticles. Historically, silver has been recognized for its antimicrobial properties but has seen reduced medical use (Basak et al. 2020).

Studies suggest that reducing silver to nano size significantly enhances its antagonistic impact. These nanoparticles in the 10-100 nm range demonstrate substantial inhibition of drug-resistant TB agents, likely due to silver's affinity for nitrogen and sulfur, abundant in the microorganisms' cell membrane proteins. Silver nanoparticles can interact with sulfur-containing amino acids inside or outside the cell membrane, affecting bacterial viability. Additionally, silver cations released from nanoparticles interact with the phosphorous in microorganism DNA, hindering replication, and with sulfur-rich proteins, deactivating enzymatic functions. These actions collectively halt microbial infectivity (Joshi et al. 2020).

The present study aims to determine the minimum inhibitory concentration (MIC) of synthesized silver nanoparticles against two TB-causing agents, *Mycobacterium bovis* and *Mycobacterium tuberculosis*, using the microplate Alamar blue assay. The authors believe this approach holds promise for improved tuberculosis diagnosis, addressing one of humanity's major threats.

METHODOLOGY:

Microbial Strain:

In this study, two distinct bacterial strains were sourced from ATCC: one reference strain each of *Mycobacterium bovis* and *Mycobacterium tuberculosis*. Additionally, ten clinical isolates of *Mycobacterium bovis* (identified by accession numbers MB 1 – MB 10) and ten clinical isolates of *Mycobacterium tuberculosis* (designated by accession numbers MT 1 – MT 10) were acquired. Among the *Mycobacterium bovis* clinical isolates, two belonged to the SB0268 type, while two of the *Mycobacterium tuberculosis* clinical isolates were of the SB0223 type.

One of the clinical isolates used in this study was a multi-drug-resistant strain of *Mycobacterium tuberculosis*. It was diagnosed through a simplified version of the indirect proportion method, using concentrations of 40 µg/ml of rifampicin, 0.2 µg/ml of isoniazid, 4 µg/ml streptomycin, and 2 µg/ml ethambutol, following established standard protocols.

Furthermore, the minimum inhibitory concentrations of rifampicin, isoniazid, streptomycin, and ethambutol were determined for the ten different clinical isolates of both *Mycobacterium bovis* and *Mycobacterium tuberculosis* using a simplified indirect proportion methodology. The minimum inhibitory concentration for streptomycin fell within the range of 6-21 µg/ml, while for ethambutol, it ranged from 0.6-21 µg/ml, and for rifampicin, it ranged from 5-16 µg/ml. All strains were consistently maintained on the Lowenstein-Jensen medium, with fresh subcultures prepared before each evaluation. The chemicals and media used in the study were sourced from Sigma Aldrich.

Synthesis of the Silver nanoparticles:

The synthesis of silver nanoparticles was accomplished using a chemical reduction method. This synthesis procedure involved the utilization of polyvinylpyrrolidone (PVP) with a molecular weight of 40,000 as a stabilizing agent, and sodium borohydride (NaBH4) as the reducing agent.

A detailed description of the synthesis process is indicated below:

1. In a flask, 0.5 ml of a 30-millimolar trisodium citrate aqueous solution was combined with 50 ml of deionized water.
2. Additionally, 0.5 ml of a 5 mM silver nitrate aqueous solution was introduced into the flask.
3. Subsequently, 0.5 ml of a freshly prepared 50 mM NaBH4 aqueous solution was swiftly added to the mixture.
4. The mixture was observed until it exhibited a light pale-yellow color.
5. After an additional 45 seconds, the aqueous solution of PVP was incorporated into the mixture.
6. The solution was further observed until it transformed into a darker yellowish color (El Hotaby et al. 2017).

Characterization of the silver nanoparticles:

The morphology and size of the synthesized nanoparticles were analyzed using a Transmission Electron Microscope (TEM) with the following specifications: Instrument Make and Model: JOEL 1210, Joel Ltd., Tokyo, Japan

The optical absorption characteristics of the colloidal silver were examined using an Ocean Optics USB2000+VIS-NIR fiber-optic spectrophotometer. Additionally, X-ray diffraction analysis of the synthesized nanoparticles was conducted. This analysis involved taking a small quantity of the nanoparticles and drying them on a quartz plate for examination.

Preparation of the silver nanoparticles:

A two-fold serial dilution of the synthesized nanoparticles was prepared across a concentration range of 0.25-250 µg/ml. This allowed for a systematic reduction in nanoparticle concentration while maintaining a consistent dilution factor.

Assessing the Minimum Inhibitory Concentration of the newly synthesized Silver Nanoparticles, Utilizing the Microplate Alamar Blue Assay

In this study, we assessed the Minimum Inhibitory Concentration (MIC) of the two different bacterial strains using the Microplate Alamar Blue Assay. Here's how it was done:

Bacterial Inoculation:

1. Bacterial inoculums from the Lowenstein-Jensen media tube were transferred into Middlebrook 7H9 broth media (5ml).
2. The 7H9 broth media contained 0.5% glycerol and 0.1% casitone, supplemented with albumin, dextrose, oleic acid, and catalase (7H9-S) from Becton Dickinson Microbiology Systems, USA.
3. A few glass beads were added to the mixture.
4. The mixture was incubated for seven days at 37±2 °C.

Sample Preparation:

1. After incubation, the tube was vortexed for 180 seconds and allowed to rest for 20 minutes.
2. The resulting supernatant was transferred to another fresh test tube.
3. The turbidity of this suspension was adjusted to a McFarland metric of 1 using a nephelometer.
4. This suspension was then diluted 1:6 in 7H9-S and used as the test inoculum.

Microplate Alamar Blue Assay:

- The assay followed a standard protocol.
- In a sterile 96-well microtitre plate (from Becton Dickinson Labware, USA), 200 µl of autoclaved de-ionized water was added.
- For each bacterial strain, 100 µl of 7H9-S containing the nanoparticle dilution was added to the wells and inoculated with 100 µl of the mycobacterial suspension in diluted form.
- The plates were sealed with parafilm and incubated for five days at 37±2 °C.
- After incubation, 25 µl of a freshly prepared 1:1 mixture of 10% Tween 80 and AB reagent was added to each well.
- The plates were further incubated for 24 hours.
- The presence of a blue color indicated no bacterial growth, while a pink color indicated bacterial growth.
- The MIC was determined as the lowest drug concentration preventing the change in color from blue to pink.

Control sets including standalone media, AB + media, drug dilution + AB + media, and bacterial cell + media + AB were included. All experiments were conducted within a biological safety cabinet (Collins et al. 1997).

RESULTS:

Characterization of the Silver Nanoparticle:

In this study, we synthesized silver nanoparticles using a chemical reduction method. The transmission electron microscopic images in Figure 1 revealed that these nanoparticles had a mean radius falling within the range of 40-60 nm. Additionally, our observations indicated that the synthesized nanoparticles predominantly exhibited spherical and tetrahedral shapes.

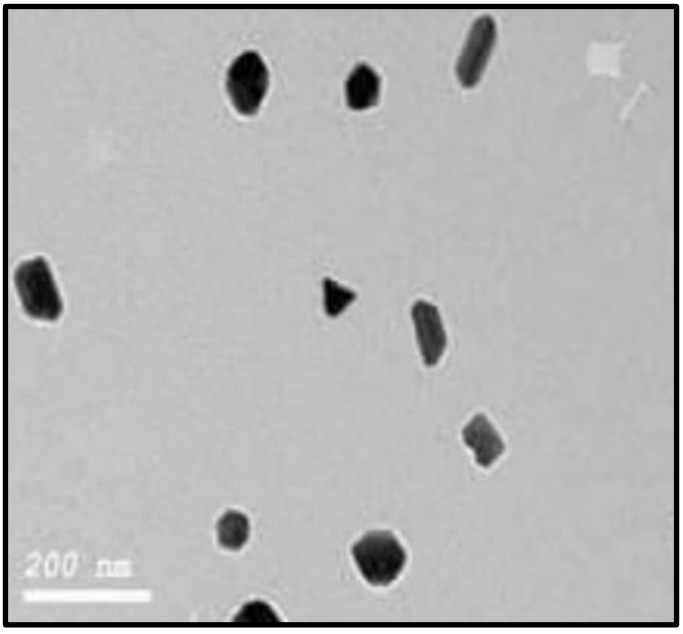

Figure 1. Photographs acquired through Transmission Emission Microscopy of the synthesized Silver Nanoparticles.

Silver nanoparticles' effects on different mycobacterial species:

To assess the effectiveness of the synthesized silver nanoparticles against the two mycobacterial strains in vitro, a range of concentrations from 0.25 µl/ml to 250 µl/ml was examined using the Minimum Inhibitory Concentration (MIC) AB assay. The results obtained have been illustrated in Figure 2. These

results unequivocally demonstrated the potent antagonistic activity of the silver nanoparticles against the two mycobacterial strains investigated in this study.

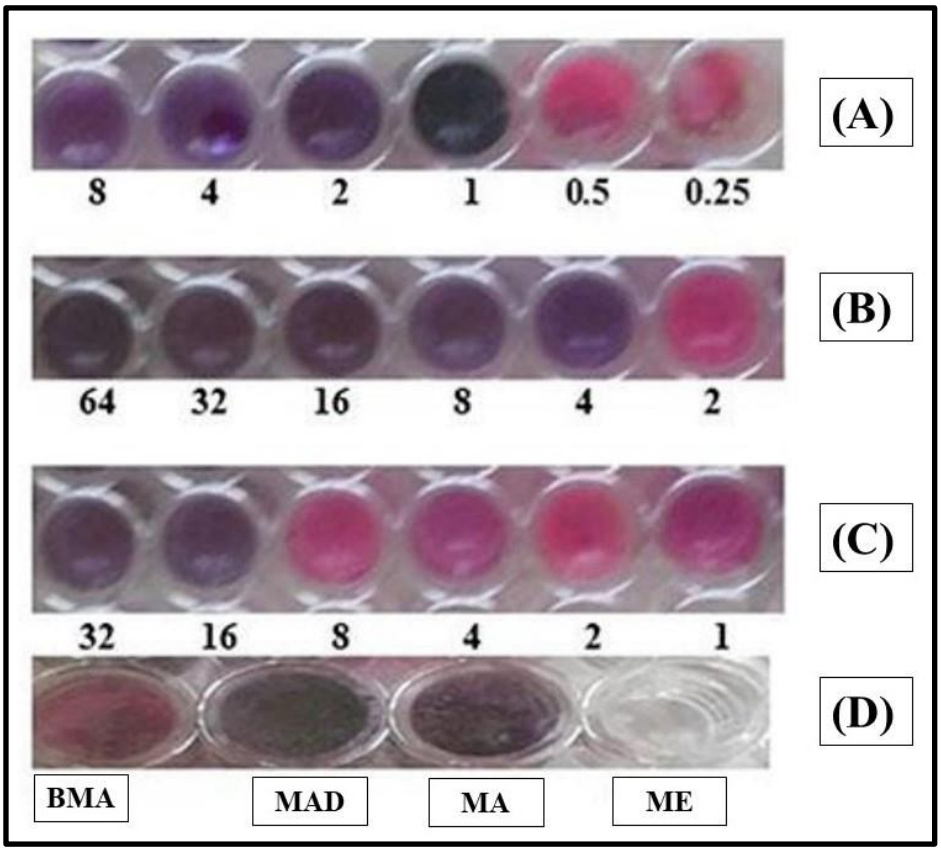

Figure 2. A) Determination of the Minimum Inhibitory Concentration (MIC) of synthesized nanoparticles (µl/ml) against Mycobacterium tuberculosis H37Rv. B) Using *Mycobacterium bovis* as a reference strain. C) Testing against Multi-Drug Resistant (MDR) *Mycobacterium tuberculosis*. D) Conducting the Alamar blue assay, including the control group.

DISCUSSION:

Antibacterial agents play a crucial role in various industries, including textiles, medicine, water purification, and food & beverage processing (Makvandi et al. 2021). Nanomaterials, in particular, have emerged as effective antimicrobial agents due to their ability to interact with and disrupt bacterial cell membranes. This disruption occurs as silver ions in the nanoparticles bind to nitrogen, oxygen, and sulfur moieties within the amino acids of the membrane proteins, leading to membrane damage and cell death (Gupta et al. 2019).

Nanoparticle-based conjugates have also shown promise in diagnosing tuberculosis. They offer solutions to challenges such as drug bioavailability, frequent dosing requirements, the emergence of multi-drug resistance, and enhanced bactericidal activity (Dahiya et al. 2020). Hence, silver nanoparticles have garnered considerable attention as a potential tool in the prevention and management of tuberculosis.

However, despite extensive research on the antagonistic effects of silver nanoparticles against various mycobacterial species, there has been a gap in investigating their impact on *Mycobacterium bovis*. This novelty underscores the significance of the present study.

The study's results revealed varying susceptibilities of different mycobacterial strains to the synthesized silver nanoparticles. The minimum inhibitory concentration (MIC) values of the nanoparticles were found to be effective against both *Mycobacterium tuberculosis* and *Mycobacterium bovis*, with MIC values of 4 µl/ml and 16 µl/ml, respectively. Interestingly, these findings suggested a higher level of antibiotic resistance in *Mycobacterium tuberculosis* compared to *Mycobacterium bovis*. Moreover, it was observed that a higher concentration of silver nanoparticles was required to neutralize *Mycobacterium bovis* compared to *Mycobacterium tuberculosis*. This implies that the synthesized silver nanoparticles possess the potential to inhibit the growth of various Mycobacterium species.

It's worth noting that the efficacy of silver nanoparticles depends on several parameters influencing their antimicrobial properties. These parameters include particle size, surface area, and the release of silver ions in aqueous solutions (Islam et al. 2018). Smaller particle size, lower surface area, and increased release of silver ions (Ag+) collectively contribute to enhanced antimicrobial efficacy.

In a recent in-vivo experiment involving 65 white mice infected with a virulent strain of Mycobacterium tuberculosis, treatment with silver nanoparticles and isoniazid resulted in a remarkable survival rate of around 95% (Krishna et al. 2017). This is particularly significant because the therapy for tuberculosis, both in animals and humans, often relies on similar drugs, which may not always be practical.

The findings of this study propose a novel approach to combating tuberculosis in both animals and humans. However, further research is warranted to elucidate the mechanism of action of silver nanoparticles and optimize various process parameters for effectively neutralizing the causative agent of tuberculosis (Dey et al. 2022; Roy et al. 2022; Roy and Ray 2022). This could be an exciting avenue for future research.

CONCLUSION:

The current study has demonstrated that the laboratory-synthesized silver nanoparticles possess significant potential for inhibiting the growth and proliferation of two distinct tuberculosis strains: *Mycobacterium bovis* and *Mycobacterium tuberculosis*. However, to translate these findings into commercial applications, further comprehensive research is necessary. It is imperative to expand the scope of this study, particularly by involving a larger sample size, in order to gain deeper insights into the true antimicrobial potential of these nanoparticles.

The authors firmly believe that this research has the potential to deliver substantial benefits to a wide range of stakeholders and could pave the way for innovative tuberculosis treatment approaches.

REFERENCES:

Basak, S., & Packirisamy, G. (2020). Nano-based antiviral coatings to combat viral infections. Nano-Structures & Nano-Objects, 24, 100620.

Collins, L. A., & Franzblau, S. G. (1997). Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against Mycobacterium tuberculosis and Mycobacterium avium. Antimicrobial agents and chemotherapy, 41(5), 1004-1009.

Dahiya, B., Sharma, S., Khan, A., Kamra, E., Mor, P., Sheoran, A., ... & Mehta, P. K. (2020). Detection of mycobacterial CFP-10 (Rv3874) protein in tuberculosis patients by gold nanoparticle-based real-time immuno-PCR. Future Microbiology, 15(8), 601-612.

Dey, P., Roy, R., Mukherjee, A., Krishna, P. S., Koijam, R., & Ray, S. (2022). Valorization of Waste Biomass as a Strategy to Alleviate Ecological Deficit: A Case Study on Waste Biomass Derived Stable Carbon. Advanced Microscopy, 167-196.

Ducati, R. G., Ruffino-Netto, A., Basso, L. A., & Santos, D. S. (2006). The resumption of consumption: a review on tuberculosis. Memórias do Instituto Oswaldo Cruz, 101, 697-714.

El Hotaby, W., Sherif, H., Hemdan, B., Khalil, W., & Khalil, S. (2017). Assessment of in situ-prepared polyvinylpyrrolidone-silver nanocomposite for antimicrobial applications. Acta Physica Polonica A, 131(6), 1554-1560.

Esch, K. J., & Petersen, C. A. (2013). Transmission and epidemiology of zoonotic protozoal diseases of companion animals. Clinical microbiology reviews, 26(1), 58-85.

Joshi, A. S., Singh, P., & Mijakovic, I. (2020). Interactions of gold and silver nanoparticles with bacterial biofilms: Molecular interactions behind inhibition and resistance. International Journal of Molecular Sciences, 21(20), 7658.

Ghosal, A., Roy, R., Sharma, K., Mitra, P., & Vora, K. (2022). Antibiofilm activity of Phytocompoundsagainst of Staphylococcus aureusBiofilm forming Protein-In silicostudy. American Journal of Applied Bio-Technology Research, 3(1), 27-29.

Gupta, A., Mumtaz, S., Li, C. H., Hussain, I., & Rotello, V. M. (2019). Combatting antibiotic-resistant bacteria using nanomaterials. Chemical Society Reviews, 48(2), 415-427.

Guzel, S., Kahraman, A., Ulger, M., Ozay, Y., Bozgeyik, I., & Sarikaya, O. (2019). Morphology, myxocarpy, mineral content and in vitro antimicrobial and antiproliferative activities of mericarps of the vulnerable Turkish endemic Salvia pilifera.

Islam, M. S., Akter, N., Rahman, M. M., Shi, C., Islam, M. T., Zeng, H., & Azam, M. S. (2018). Mussel-inspired immobilization of silver nanoparticles toward antimicrobial cellulose paper. ACS Sustainable Chemistry & Engineering, 6(7), 9178-9188.

Krishna, P. G., Ananthaswamy, P. P., Trivedi, P., Chaturvedi, V., Mutta, N. B., Sannaiah, A., ... & Yadavalli, T. (2017). Antitubercular activity of ZnO nanoparticles prepared by solution combustion synthesis using lemon juice as bio-fuel. Materials science and Engineering: C, 75, 1026-1033.

Makvandi, P., Iftekhar, S., Pizzetti, F., Zarepour, A., Zare, E. N., Ashrafizadeh, M., ... & Rossi, F. (2021). Functionalization of polymers and nanomaterials for water treatment, food packaging, textile and biomedical applications: A review. Environmental Chemistry Letters, 19(1), 583-611.

Raza, S., Ansari, A., Siddiqui, N. N., Ibrahim, F., Abro, M. I., & Aman, A. (2021). Biosynthesis of silver nanoparticles for the fabrication of non cytotoxic and antibacterial metallic polymer based nanocomposite system. Scientific Reports, 11(1), 1-15.

Reddington, K., O'Grady, J., Dorai-Raj, S., Niemann, S., van Soolingen, D., & Barry, T. (2011). A novel multiplex real-time PCR for the identification of mycobacteria associated with zoonotic tuberculosis. PLoS One, 6(8), e23481.

Roy, R., Debnath, D., & Ray, S. (2022). Comprehensive Assessment of Various Lignocellulosic Biomasses for Energy Recovery in a Hybrid Energy System. Arabian Journal for Science and Engineering, 47(5), 5935-5948.

Roy, R., & Ray, S. (2019). Effect of various pretreatments on energy recovery from waste biomass. Energy Sources, Part A: Recovery, Utilization, and Environmental Effects, 1-13.

Roy, R., & Ray, S. (2020). Development of a non-linear model for prediction of higher heating value from the proximate composition of lignocellulosic biomass. Energy Sources, Part A: Recovery, Utilization, and Environmental Effects, 1-14.

Roy, R., & Ray, S. (2022). Upgradation of an Agro-residue by Acid Pretreatment into a Solid Fuel with Improved Energy Recovery Potential: An Optimization Study. Arabian Journal for Science and Engineering, 47(5), 6311-6323.

Roy, R., Sarkar, S., Kotak, R., Nandi, D., Shil, S., Singha, S., ... & Tarafdar, S. (2022). Evaluation of the Water Quality Parameters from Different Point Sources: A Case Study of West Bengal. American Journal of Applied Bio-Technology Research, 3(3), 18-28.

Roy, R., Shil, S., Choudhary, D. K., Mondal, P., Adhikary, P., Manna, U., ... & Maji, M. (2022). Conversion of glucose into calcium gluconate and determining the process feasibility for further scaling-up: An optimization approach. Int. J. Exp. Res. Rev, 27, 1-10.

Roy, R., Srinivasan, A., Bardhan, S., & Paul, T. Evaluation of the Expression of CD-4 and CD-45 Count among Patients Having Non-Small Cell Lung Cancer. Journal homepage: www. ijrpr. com ISSN, 2582, 7421.

Song, B., Liu, X., Dong, H., & Roy, R. (2022). miR-140-3P Induces Chemotherapy Resistance in Esophageal Carcinoma by Targeting the NFYA-MDR1 Axis. Applied Biochemistry and Biotechnology, 1-19.

Su, Q., Dong, J., Zhang, D., Yang, L., & Roy, R. (2022). Protective effects of the bilobalide on retinal oxidative stress and inflammation in streptozotocin-induced diabetic rats. Applied Biochemistry and Biotechnology, 1-16.

Vipparla, C., Sarkar, S., Manasa, B., Pattela, T., Nagari, D. C., Aradhyula, T. V., & Roy, R. (2022). Enzyme Technology in Biofuel Production. In Bio-Clean Energy Technologies Volume 2 (pp. 239-257). Springer, Singapore.

World Health Organization. (2016). World health statistics 2016: monitoring health for the SDGs sustainable development goals. World Health Organization.