Review Form 3

Journal Name:	**International Journal of Biochemistry Research & Review**
Manuscript Number:	**Ms_IJBCRR_124324**
Title of the Manuscript:	**Elephantorrhiza elephantina mediated biosynthesised AgNPs, characterisation and bioactivity against surrogate model Mycobacterium tuberculosis species**
Type of the Article	**Original Research Article**

Review Form 3

PART 1: Review Comments

Compulsory REVISION comments	**Reviewer's comment**	**Author's Feedback** *(Please correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Please write a few sentences regarding the importance of this manuscript for the scientific community. Why do you like (or dislike) this manuscript? A minimum of 3-4 sentences may be required for this part.	This manuscript is significant for the scientific community as it explores the use of Elephantorrhiza elephantina for the biosynthesis of silver nanoparticles (AgNPs), presenting an eco-friendly and sustainable method for nanoparticle production. The characterization of these AgNPs, along with their bioactivity against a surrogate model of Mycobacterium tuberculosis, offers valuable insights into alternative approaches to combat tuberculosis. The interdisciplinary nature of this work, bridging plant-based synthesis, nanotechnology, and microbiology, is particularly appealing. I appreciate how the study advances both green synthesis methods and potential therapeutic applications, making it relevant for researchers in drug development and infectious disease control.	
Is the title of the article suitable? **(If not please suggest an alternative title)**	The title of the article is informative but could be slightly refined for clarity and impact. It currently conveys the key elements but might benefit from a more concise phrasing. An alternative title could be: "Green Synthesis of Silver Nanoparticles Using Elephantorrhiza elephantina and Their Antimicrobial Activity Against Mycobacterium tuberculosis Surrogate Models." This version highlights the core aspects—green synthesis, AgNPs, and antimicrobial activity—while improving readability.	
Is the abstract of the article comprehensive? Do you suggest the addition (or deletion) of some points in this section? Please write your suggestions here.	The abstract is comprehensive and well-structured, providing a clear overview of the study's purpose, methods, and findings. However, a few minor adjustments could improve its clarity and flow: 1. Objective clarity: The purpose of the study could be slightly more specific by mentioning the role of silver nanoparticles (AgNPs) early in the sentence. For example, "The purpose of this study was to biosynthesize and characterize silver nanoparticles (AgNPs) using Elephantorrhiza elephantina, and to evaluate their antimicrobial effectiveness against surrogate Mycobacterium tuberculosis species." 2. Methodology details: The mention of "various phyto-screening techniques" could be elaborated to briefly mention which techniques were used, or simply revised for brevity, e.g., "Phytochemical screening confirmed the presence of bioactive compounds..." 3. Results refinement:The specific mention of the structural morphology (spherical and cubic) and average nanoparticle size is useful, but the presentation could be smoother by combining related points: "Transmission electron microscopy and dynamic light scattering confirmed the AgNPs to have an average size of 44 nm, with spherical and cubic morphologies." 4. Conclusion emphasis: The conclusion could more strongly emphasize potential impact of the findings: "The study concluded that biosynthesized AgNPs from E. elephantina root extract using a one-pot green-synthesis technique were both safe and effective, offering a promising alternative therapeutic strategy for combating multidrug-resistant tuberculosis." These refinements would enhance the abstract's clarity without removing any critical information.	
Are subsections and structure of the manuscript appropriate?	The subsections and structure of the manuscript appear to be appropriate based on the abstract provided. The typical structure of a research manuscript includes the following subsections: 1. Introduction: Provides background on the problem (MDR and XDR Mycobacterium tuberculosis), the significance of the study, and the rationale for using *Elephantorrhiza elephantina* and silver nanoparticles (AgNPs) in tuberculosis treatment.	

	2. Materials and Methods: Details the synthesis process of AgNPs, phytochemical screening techniques, characterization methods (UV-VIS spectrometry, TEM, DLS), and toxicity testing following OECD guidelines. This section should also describe how bioactivity against *Mycobacterium tuberculosis surrogate models was evaluated. 3. Results: Presents the findings, such as phytochemical screening outcomes, nanoparticle characterization (size, morphology), and antimicrobial and toxicity data. Numerical results, such as MIC values and inhibition zones, should be clearly outlined. 4. Discussion: Interprets the results in the context of current literature, highlighting the relevance of AgNPs synthesized using E. elephantina in tuberculosis treatment. The discussion should also mention the potential implications of the findings for developing antimicrobials and how these results compare with previous studies. 5. Conclusion: Summarizes the key findings and emphasizes the potential application of biosynthesized AgNPs as an alternative therapy for MDR-TB. 6. References: Cites all relevant literature used in the research and discussion sections. If the manuscript follows this structure, it should be appropriate. The division into clear subsections helps maintain logical flow and ensures that all critical aspects of the research are addressed. However, reviewing the full manuscript would confirm if these sections are well-balanced and cohesive.	
Please write a few sentences regarding the scientific correctness of this manuscript. Why do you think that this manuscript is scientifically robust and technically sound? A minimum of 3-4 sentences may be required for this part.	This manuscript appears to be scientifically robust and technically sound due to its comprehensive approach to biosynthesizing and characterizing silver nanoparticles (AgNPs) using Elephantorrhiza elephantina. The use of multiple, well-established techniques such as UV-VIS spectrometry, transmission electron microscopy (TEM), and dynamic light scattering (DLS) ensures accurate characterization of the nanoparticles in terms of their structure, size, and morphology. Additionally, the antimicrobial efficacy was rigorously tested against surrogate *Mycobacterium tuberculosis species, providing valuable data on the potential therapeutic application. The in vivo toxicity evaluation following OECD guidelines adds further validity by confirming the safety profile of the biosynthesized AgNPs. This thorough methodology supports the scientific credibility and potential of the study's findings.	
Are the references sufficient and recent? If you have suggestions of additional references, please mention them in the review form. -	These references would help the authors to incorporate the more information on Nanoparticles **https://doi.org/10.1007/s42452-024-05897-z**, **https://doi.org/10.1093/rpsppr/rqae015**, DOI: 10.17344/acsi.2023.8216.	

Review Form 3

Minor REVISION comments **Is the language/English quality of the article suitable for scholarly communications?**	The language and English quality of the article, based on the abstract provided, seem generally suitable for scholarly communication. The manuscript uses appropriate technical terminology and clearly conveys the research objectives, methods, and findings. However, some sentences could be refined for clarity and flow. For example, simplifying complex sentences and ensuring smooth transitions between ideas would enhance readability. Minor grammatical improvements, such as rewording or condensing certain sections, could also improve the overall presentation. Overall, the English quality is adequate but could benefit from minor editing to improve precision and readability, making it more polished for a scholarly audience.	
Optional/General comments	Here are t comments regarding the provided paragraphs on Elephantorrhiza elephantina and antibacterial resistance: 1. Clarity and Flow: The paragraphs provide a comprehensive overview of *E. elephantina* and its therapeutic uses, but some sentences could be simplified for better clarity and flow. For example, consider breaking up longer sentences to enhance readability. 2. Use of Citations: The references (e.g., [1–3], [4-6]) should be checked for consistency in formatting and alignment with the journal's citation style. Ensure that all cited works are listed in the references section. 3. Contextual Background: The introduction effectively sets the context for the use of *E. elephantina* in traditional medicine. However, providing specific examples of how this plant is used in various cultures could further enrich the discussion. 4. Scientific Evidence: While the text mentions the presence of bioactive phyto-compounds, it would be beneficial to include specific studies or data that highlight the antimicrobial activity of these compounds, particularly against *Mycobacterium tuberculosis*. 5. Connection to Antimicrobial Resistance: The transition from discussing E. elephantina*to the topic of antimicrobial resistance is smooth, but it could be strengthened by explicitly linking the plant's properties to its potential role in combating drug-resistant infections. 6. Current Statistics: The statistics regarding drug-resistant tuberculosis treatment success rates (54-65%) are relevant. However, including more recent data or trends would provide a more updated perspective on the issue. 7. Emerging Resistance Mechanisms: The paragraph discussing mechanisms of antimicrobial evasion is well articulated. Consider providing examples of specific pathogens and their resistance mechanisms to give readers a clearer understanding. 8. Plant Phytochemicals: The mention of plant phytochemicals as an alternative strategy to combat antimicrobial resistance is promising. Elaborating on specific phytochemicals found in *E. elephantina* and their mechanisms of action could enhance this point. 9. Impact of New Drugs: The discussion on the BPaL regimen and its associated side effects is pertinent. It would be helpful to briefly mention alternative treatment options or ongoing research into new antimicrobials to provide a more rounded view of the treatment landscape. 10. Future Directions: The concluding remarks could suggest potential future research directions, such as the development of formulations combining *E. elephantina* extracts with conventional antibiotics, or clinical trials investigating its effectiveness against MDR and XDR strains of tuberculosis. This could inspire further inquiry and application of the findings discussed.	

	Here are 15 questions for the authors based on the provided sections of the manuscript: General Questions: 1. Objective Clarification: What specific objectives did you aim to achieve with the preparation and characterization of E. elephantina extracts and AgNPs? Section 2.1: Materials, Equipment, and Facilities 2. Equipment Details: Can you provide more details about the specific equipment used for the characterization of AgNPs and how their specifications contribute to the accuracy of the results? 3. Source of Microorganisms: How were the test microorganisms sourced, and what criteria were used to select the specific strains of *Mycobacterium tuberculosis* for this study? Section 2.2: Hydro-Methanolic Extract Preparation and Characterization 4. Extraction Method: Why did you choose the hydro-methanolic extraction method over other possible methods, and what advantages does it provide in terms of phytochemical yield? 5. Phytochemical Screening: Can you elaborate on the qualitative phytochemical screening methods used? What specific compounds were you aiming to identify? Section 2.2.1: Qualitative Phytochemical Screening 6. Result Interpretation: How did the results of the qualitative phytochemical screening correlate with the known therapeutic properties of *E. elephantina*? Section 2.2.2: Quantification of Total Flavonoid and Tannins 7. Quantification Techniques: What specific methods were used for the quantification of flavonoids and tannins, and how were the results validated? Section 2.3: Acute Oral Toxicity Testing 8. Toxicity Testing Protocol: Can you describe the acute oral toxicity testing protocol used, including the choice of animal model and dosage determination? 9. Safety Threshold: How did you determine that the LD50 of the lyophilized *E. elephantina* extract is safe above 4000 mg/kg body weight, and what implications does this have for future studies? Section 2.4: Biosynthesis of Silver Nanoparticles 10. **Biosynthesis Process: What specific conditions (e.g., temperature, pH, reaction time) were optimized during the biosynthesis of AgNPs, and how did these conditions affect the yield and properties of the nanoparticles? Section 2.4.1: Characterization of the Biosynthesized AgNPs 11. Characterization Techniques: Could you detail the characterization techniques employed to analyze the size, shape, and surface charge of the synthesized AgNPs? Section 2.5: Determination of Antimycobacterial Activity 12. Antimycobacterial Assays: What controls were implemented during the determination of antimycobacterial activity, and how do you ensure the reliability of the MIC and zone of inhibition results? Section 2.5.1: Preparation of Plant Extracts 13. Standardization of Extracts: How did you standardize the plant extracts before testing for antimycobacterial activity to ensure consistency across different experiments? Section 2.5.2: Determination of Minimum Inhibitory Concentration (MIC) 14. MIC Testing Conditions: What specific conditions (e.g., incubation time, temperature, medium) were used for MIC determination, and how were they optimized for this study? Section 2.5.3: Zone of Inhibition Test	

	15. Interpretation of Results: How do you interpret the inhibition zone results in the context of potential clinical applications for the biosynthesized AgNPs, and what further studies do you envision based on these findings? These questions aim to gain a deeper understanding of the methods and findings presented in the manuscript, as well as to clarify the rationale behind the experimental designs.	

PART 2:

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Are there ethical issues in this manuscript?	*(If yes, Kindly please write down the ethical issues here in details)*	

Reviewer Details:

Name:	**Sarad Pawar Naik Bukke**
Department, University & Country	**Kampala International University, Uganda**