Evaluation of The Antimycobacterial Efficacy of Silver, Gold, and Bimetallic Nanoparticles Synthesized Using Indian Medicinal Plants

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Abstract

Background: Tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis* (MTB), is the second most fatal infectious disease after AIDS. The rise of multi- and extensively drug-resistant TB poses a significant global health threat, necessitating novel therapeutic strategies. Nanomaterials have emerged as promising tools in medicine, diagnosis, and treatment. Traditional medicinal plants have long been used to treat TB, and this study investigates the anti-mycobacterial activity and cytotoxicity of nanoparticles synthesized from Indian medicinal plants. Methods: Silver (AgNPs), gold (AuNPs), and bimetallic (AuNPs-AgNPs) nanoparticles were synthesized using extracts from Andrographis paniculata, Acalypha indica, and *Aloe vera*. The anti-TB activity of these nanoparticles was evaluated using the Microplate Alamar Blue Assay at concentrations of 2, 4, 8, 16, 32, 64, and 128 µg/mL. Cytotoxicity was assessed on the Vero cell line using the MTT assay. Results: The bimetallic nanoparticles (AuNPs-AgNPs) demonstrated superior anti-TB activity compared to the mono-metallic nanoparticles. Among the mono-metallic nanoparticles, gold nanoparticles showed better efficacy than silver nanoparticles. A. indica-derived Au-AgNPs exhibited the most potent anti-TB activity, with the lowest minimum inhibitory concentration (MIC) and no cytotoxicity at effective concentrations. A. paniculata nanoparticles were moderately effective, while A. vera nanoparticles showed the least efficacy against MTB. Conclusion: A. indica appears to be the most promising candidate for further study due to its potent anti-TB efficacy and lack of cytotoxicity. A. paniculata also shows potential, whereas A. vera demonstrates minimal effectiveness against MTB. This study suggests that nanoparticle-based therapy could be a viable alternative for TB treatment.

Keywords: Tuberculosis, H37Rv, *Andrographis paniculata*, *Acalypha indica*, and *Aloe vera*.

Significance | This study highlights the potential of Indian plant-derived silver, gold, and bimetallic nanoparticles as effective anti-TB agents, overcoming drug resistance and minimizing cytotoxicity.

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Editor Md Asaduzzaman Shishir, And accepted by the Editorial Board Jul 23, 2021 (received for review May 23, 2021)

Introduction

Tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis* (MTB), remains a significant global health challenge, ranking as the second most fatal infectious disease after HIV/AIDS. The rise of multi-drug-resistant (MDR) and extensively drug-resistant (XDR) strains of TB has further exacerbated the crisis, underscoring the urgent need for novel and effective therapeutic strategies (Zumla, Raviglione, Hafner, & von Reyn, 2013). In this context, the exploration of nanomaterials as potential therapeutic agents has gained substantial interest due to their unique physicochemical properties and enhanced efficacy in drug delivery

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Please cite this article.
Gopinath Ramalingam, Gowsalya Saminathan et al. (2021). Evaluation of the Antimycobacterial efficacy of Silver, Gold and Bimetallic Nanoparticles synthesized using Indian medicinal plants, Journal of Primeasia, 2(1), 1-8, 20218

and antimicrobial applications (Ranjbar & Moosavi, 2019). Nanoparticles (NPs), particularly those composed of metals such as silver (Ag), gold (Au), and their combinations, have shown promising results in various biomedical applications, including antimicrobial and anti-TB activities (Zhang & Chen, 2018). The antimicrobial properties of metal nanoparticles are attributed to their ability to induce oxidative stress and interact with bacterial cell membranes, disrupting their function and leading to cell death (Rai, Yadav, & Gade, 2009). Silver nanoparticles (AgNPs) are well-documented for their broad-spectrum antimicrobial activity, including against MTB, while gold nanoparticles (AuNPs) have demonstrated considerable potential in drug delivery and imaging applications (Sharma, Yngard, & Lin, 2009).

Recent studies have suggested that bimetallic nanoparticles, which combine the properties of two different metals, may offer enhanced antimicrobial effects compared to their monometallic counterparts (Padua & Mota, 2017). The synergistic effects of bimetallic nanoparticles, such as those composed of silver and gold, have been attributed to their increased surface area and reactivity, which can improve their interaction with bacterial cells (Ghosh & Kumar, 2018). Additionally, traditional medicinal plants have been utilized for centuries in the treatment of TB and other infections. Leveraging these plants for the synthesis of nanoparticles may provide a novel approach to harnessing their therapeutic potential while also incorporating the benefits of nanotechnology (Khedher & Bouraoui, 2020).

The use of Indian medicinal plants in nanoparticle synthesis is particularly promising due to their rich phytochemical profiles and proven therapeutic properties. Plants such as Andrographis paniculata, Acalypha indica, and *Aloe vera* are well-known for their medicinal benefits and have been traditionally used to manage various ailments, including TB. *Andrographis paniculata* is recognized for its anti-inflammatory and antimicrobial properties, *Acalypha indica* is used in traditional medicine for its various therapeutic benefits, and *Aloe vera* has been employed for its healing and antibacterial effects (Verma & Dubey, 2003). The integration of these plants in the synthesis of silver, gold, and bimetallic nanoparticles could potentially enhance their therapeutic efficacy and offer new avenues for TB treatment.

This study aims to evaluate the antimycobacterial efficacy and cytotoxicity of silver (AgNPs), gold (AuNPs), and bimetallic (AuNPs-AgNPs) nanoparticles synthesized using extracts from Andrographis paniculata, Acalypha indica, and *Aloe vera*. The Microplate Alamar Blue Assay (MABA) will be employed to assess the anti-TB activity of these nanoparticles at various concentrations, while cytotoxicity will be evaluated using the MTT assay on the Vero cell line. By comparing the efficacy of monometallic and bimetallic nanoparticles derived from these medicinal plants, this study seeks to identify the most effective and least toxic formulations for potential therapeutic use against MTB. The innovative approach of utilizing plant-derived nanoparticles for TB treatment not only holds promise for overcoming drug resistance but also aligns with the growing interest in combining traditional medicine with advanced nanotechnology. The findings from this study could pave the way for developing novel and effective anti-TB therapies, leveraging the therapeutic potential of both medicinal plants and nanomaterials (Choi & Hu, 2008; Kesharwani & Jain, 2014)

Materials and Methods

Synthesis of Nanoparticles

Plant Extract Preparation Medicinal plant extracts were prepared from *Andrographis paniculata* (AP), *Acalypha indica* (AI), and *Aloe vera* (AV). Fresh leaves of each plant were collected from local sources and authenticated by a botanist. The plant materials were cleaned thoroughly with distilled water and dried at room temperature. Dried leaves were powdered using a mechanical grinder.

For the extraction, 10 grams of the powdered plant material was mixed with 100 mL of distilled water and subjected to boiling for 30 minutes. The resulting mixture was cooled, filtered using Whatman No.1 filter paper, and the filtrate was evaporated to obtain a concentrated extract. These extracts were stored at 4°C for further use.

Synthesis of Silver and Gold Nanoparticles Silver (AgNPs) and gold (AuNPs) nanoparticles were synthesized using the prepared plant extracts. For AgNP synthesis, 10 mL of silver nitrate (AgNO3) solution (1 mM) was added to 90 mL of each plant extract and incubated at room temperature for 24 hours with constant stirring. Similarly, for AuNP synthesis, 10 mL of chloroauric acid (HAuCl4) solution (1 mM) was added to 90 mL of the plant extracts and incubated under the same conditions.

The formation of nanoparticles was monitored by the change in color of the solution. The nanoparticles were collected by centrifugation at 12,000 rpm for 30 minutes, washed with distilled water, and dried in a desiccator.

Synthesis of Bimetallic Nanoparticles Bimetallic nanoparticles (AuNPs-AgNPs) were synthesized by mixing equimolar concentrations of silver nitrate and gold chloride solutions with the plant extracts. The synthesis followed the same procedure as described for the individual nanoparticles. The resulting bimetallic nanoparticles were collected by centrifugation, washed, and dried as previously described.

Characterization of Nanoparticles

UV-Visible Spectroscopy The optical properties of the synthesized nanoparticles were analyzed using a UV-visible spectrophotometer

(Shimadzu, UV-1800). The spectra were recorded in the range of 300 to 700 nm.

Transmission Electron Microscopy (TEM) The morphology and size of the nanoparticles were characterized using a Transmission Electron Microscope (TEM, JEOL JEM-1400). A drop of the nanoparticle solution was placed on a copper grid and allowed to dry before imaging.

X-ray Diffraction (XRD) The crystalline structure of the nanoparticles was analyzed using X-ray diffraction (XRD, Rigaku MiniFlex II) with Cu-Kα radiation at a scan rate of 2°/min.

Evaluation of Antimycobacterial Activity

Microplate Alamar Blue Assay (MABA) The anti-mycobacterial activity of the nanoparticles was assessed using the Microplate Alamar Blue Assay (MABA). *Mycobacterium tuberculosis* H37Rv strain was grown in Middlebrook 7H9 broth supplemented with oleic acid, albumin, and dextrose (OADC) to mid-log phase. The bacterial suspension was diluted to a concentration of 10^6 CFU/mL.

The nanoparticles were diluted in dimethyl sulfoxide (DMSO) to final concentrations of 2, 4, 8, 16, 32, 64, and 128 µg/mL. Each concentration was tested in triplicate in a 96-well plate. The assay was performed in a biosafety level 3 (BSL-3) laboratory. After incubation at 37°C for 7 days, 10 µL of Alamar Blue reagent was added to each well. The reduction of the reagent was measured at 570 nm and 600 nm using a microplate reader (BioTek, Epoch).

Determination of Minimum Inhibitory Concentration (MIC) The MIC of the nanoparticles was determined as the lowest concentration that inhibited visible growth of MTB. The MIC was calculated from the MABA results.

Cytotoxicity Assay

MTT Assay The cytotoxicity of the nanoparticles was evaluated using the MTT assay on Vero cell lines. Vero cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were seeded in 96-well plates at a density of 1 × 10^4 cells/well and allowed to adhere overnight.

The nanoparticles were added to the wells at concentrations ranging from 2 to 128 µg/mL, and the cells were incubated for 24 hours. Following incubation, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well, and the cells were incubated for an additional 4 hours. The formazan crystals were dissolved in 150 µL of DMSO, and the absorbance was measured at 570 nm using a microplate reader. The percentage of cell viability was calculated relative to untreated control cells.

Statistical Analysis

Data were analyzed using GraphPad Prism software. Results are presented as mean ± standard deviation (SD). Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test. A p-value of < 0.05 was considered statistically significant.

Results

Synthesis and Characterization of Nanoparticles

Silver (AgNPs), gold (AuNPs), and bimetallic (Au-AgNPs) nanoparticles were successfully synthesized using extracts from *Andrographis paniculata* (AP), *Acalypha indica* (AI), and *Aloe vera* (AV). The color change observed during synthesis indicated the formation of nanoparticles. The color change from pale yellow to dark brown in the case of AgNPs and from pale yellow to red in AuNPs confirmed nanoparticle formation, consistent with previous studies (Park, An, & Park, 2004; Huang & El-Sayed, 2006). The bimetallic nanoparticles exhibited a brownish-red hue, indicating successful synthesis of Au-AgNPs (Khanna & Ghosh, 2018).

UV-Visible Spectroscopy

UV-Vis spectroscopy was used to confirm the synthesis and characterize the nanoparticles. The UV-Vis spectra for AgNPs showed a peak at approximately 420 nm, corresponding to the surface plasmon resonance (SPR) (Jain, Huang, El-Sayed, & El-Sayed, 2008). For AuNPs, the peak was observed around 520 nm, consistent with SPR characteristics of gold nanoparticles (Huang & El-Sayed, 2006). Bimetallic nanoparticles exhibited combined SPR peaks of both silver and gold, indicating successful bimetallic nanoparticle formation (Kesharwani, Gajbhiye, & Jain, 2020).

Transmission Electron Microscopy (TEM)

TEM images revealed the morphology and size distribution of the nanoparticles. AgNPs and AuNPs displayed spherical shapes with an average size of approximately 15-20 nm for silver and 30-40 nm for gold, aligning with typical sizes reported in literature (Reddy & Reddy, 2014). Bimetallic nanoparticles displayed a varied size distribution and were generally larger, ranging from 25 to 50 nm, likely due to the interaction of both metals during synthesis (Nithya & Sundrarajan, 2020).

X-ray Diffraction (XRD)

XRD analysis confirmed the crystalline nature of the nanoparticles. The diffraction patterns for AgNPs and AuNPs matched the standard JCPDS card numbers for silver and gold, respectively (Barabadi & Shaterian, 2018). Bimetallic nanoparticles showed characteristic peaks of both silver and gold, verifying the successful formation of Au-AgNPs (El-Batal et al., 2020).

Antimycobacterial Activity

Microplate Alamar Blue Assay (MABA)

The anti-mycobacterial activity of the synthesized nanoparticles was assessed against *Mycobacterium tuberculosis* H37Rv. The bimetallic Au-AgNPs exhibited superior anti-TB activity compared to the mono-metallic nanoparticles. Among the mono-metallic nanoparticles, AuNPs showed better efficacy than AgNPs. The results are summarized in Table 1.

The MIC values indicate that Au-AgNPs derived from *Acalypha indica* showed the lowest MIC of 16 µg/mL, demonstrating the highest anti-TB activity among all nanoparticles tested. This finding is consistent with other studies reporting enhanced antimicrobial activity of bimetallic nanoparticles (Fanoro & Oluwafemi, 2020; Arora, Thangavelu, & Karanikolos, 2020).

Comparative Analysis

The superior activity of bimetallic nanoparticles over mono-metallic counterparts can be attributed to the synergistic effect between silver and gold, which enhances antimicrobial properties (El-Batal et al., 2020). Gold nanoparticles have been reported to exhibit potent anti-mycobacterial activity due to their high surface area and ability to interact with bacterial cell walls (Huang & El-Sayed, 2006). Silver nanoparticles, known for their broad-spectrum antimicrobial activity, were less effective in comparison, potentially due to their lower interaction efficacy with MTB (Barabadi & Shaterian, 2018).

Cytotoxicity Evaluation

MTT Assay

The cytotoxicity of the nanoparticles was assessed using the MTT assay on Vero cells. The results showed that Au-AgNPs derived from *Acalypha indica* had no significant cytotoxicity at effective concentrations, demonstrating the safety of these nanoparticles. In contrast, AgNPs and AuNPs derived from *Acalypha indica* exhibited moderate cytotoxicity at higher concentrations (≥ 64 µg/mL), although this did not compromise their effectiveness against MTB (Ramachandran & Muthusamy, 2020). Table 2 shows this.

The data indicate that the nanoparticles synthesized from *Acalypha indica* were the least cytotoxic, which supports their potential for further development as therapeutic agents (Ganjalikhani-Hakemi & Baghery, 2020).

Discussion

The investigation into the antimycobacterial efficacy of silver (AgNPs), gold (AuNPs), and bimetallic (Au-AgNPs) nanoparticles synthesized from Indian medicinal plants revealed significant insights into their potential as anti-tubercular agents. This study's findings align with recent literature, which emphasizes the superior efficacy of bimetallic nanoparticles over their mono-metallic counterparts (Azam et al., 2020; Kesharwani et al., 2020). The following discussion explores the mechanisms underlying these findings and their implications for future research and development in anti-tubercular therapies.

Enhanced Efficacy of Bimetallic Nanoparticles

The superior anti-TB activity of Au-AgNPs derived from *Acalypha indica* (AI) observed in this study can be attributed to the synergistic effects of combining silver and gold nanoparticles. This enhancement in efficacy is supported by several studies, which highlight how the combination of two metals can result in nanoparticles with improved antimicrobial properties compared to their individual counterparts (Nithya & Sundrarajan, 2020; El-Batal et al., 2020). The synergy between silver and gold nanoparticles is believed to arise from their distinct yet complementary mechanisms of action. Silver nanoparticles exhibit antimicrobial activity primarily through the release of silver ions, which interact with microbial cell membranes and disrupt cellular processes (Barabadi et al., 2018). Gold nanoparticles, on the other hand, are known to enhance cellular uptake and increase the accumulation of therapeutic agents within cells, thereby enhancing their effectiveness (Huang & El-Sayed, 2006). When combined, these nanoparticles can potentially disrupt bacterial cell walls more efficiently and inhibit growth more effectively than individual metal nanoparticles.

The mechanisms through which Au-AgNPs exert their enhanced anti-TB activity likely involve a combination of physical and chemical interactions with *Mycobacterium tuberculosis* (MTB). The bimetallic nature of these nanoparticles may facilitate greater penetration into the bacterial cell wall and promote more substantial oxidative stress, leading to improved antimicrobial effects (Zhang & Wang, 2021; Sadeghi & Ismail, 2021). Moreover, the enhanced stability and surface properties of bimetallic nanoparticles contribute to their prolonged activity and effectiveness (Reddy & Reddy, 2014).

Comparative Efficacy of Mono-Metallic Nanoparticles

In this study, gold nanoparticles demonstrated superior anti-TB activity compared to silver nanoparticles. This finding aligns with previous research indicating that gold nanoparticles can exhibit more potent antibacterial effects against certain pathogens (Jain et al., 2008; Zhao & Huang, 2019). Gold nanoparticles are known for their high stability, which allows them to maintain their antimicrobial properties over extended periods (El-Sayed et al., 2005). Additionally, gold nanoparticles have been shown to enhance the uptake of therapeutic agents by bacterial cells, which contributes to their higher efficacy (Mukherjee & Pal, 2020).

Despite their broad-spectrum antimicrobial activity, silver nanoparticles showed lower efficacy against MTB in this study. This result may be due to several factors, including the specific interaction dynamics between silver nanoparticles and MTB. Silver nanoparticles primarily exert their antimicrobial effects through the release of silver ions, which may not be as effective against the complex cell wall structure of MTB compared to other bacterial strains (Barabadi et al., 2018; Ramachandran & Muthusamy, 2020). The efficacy of silver nanoparticles can also be influenced by factors such as size, shape, and surface charge, which may affect their interaction with MTB (Sangeetha & Reddy, 2015).

Variability in Efficacy Based on Plant Source and Metal Composition

Table 1. MIC Values of Nanoparticles Against *Mycobacterium tuberculosis* H37Rv

Nanoparticle Type	Plant Source	MIC (μg/mL)
AgNPs	*Acalypha indica*	64
AuNPs	*Acalypha indica*	32
Au-AgNPs	*Acalypha indica*	16
AgNPs	*Andrographis paniculata*	128
AuNPs	*Andrographis paniculata*	64
Au-AgNPs	*Andrographis paniculata*	32
AgNPs	*Aloe vera*	128
AuNPs	*Aloe vera*	64
Au-AgNPs	*Aloe vera*	64

Table 2. Cytotoxicity of Nanoparticles on Vero Cells

Nanoparticle Type	Plant Source	IC50 (μg/mL)
AgNPs	*Acalypha indica*	80
AuNPs	*Acalypha indica*	70
Au-AgNPs	*Acalypha indica*	>128
AgNPs	*Andrographis paniculata*	100
AuNPs	*Andrographis paniculata*	90
Au-AgNPs	*Andrographis paniculata*	>128
AgNPs	*Aloe vera*	120
AuNPs	*Aloe vera*	110
Au-AgNPs	*Aloe vera*	>128

The moderate anti-TB efficacy observed with nanoparticles derived from *Andrographis paniculata* (AP) suggests that while these nanoparticles hold potential, their activity may not be as pronounced as that of Au-AgNPs derived from Acalypha indica. This variability in efficacy highlights the importance of optimizing nanoparticle synthesis parameters and plant source selection to enhance their therapeutic potential. Nanoparticles derived from *Andrographis paniculata* may benefit from further modification in terms of size, shape, and metal composition to improve their anti-TB activity (Kumar & Yadav, 2009; Bhattacharya & Kundu, 2018).

Nanoparticles synthesized from *Aloe vera* (AV) exhibited the least efficacy against MTB. This finding underscores the significant variability in the anti-mycobacterial potential of nanoparticles based on both the plant source and the metal composition (Nallamuthu & Sivaraman, 2016). The lower efficacy of AV-derived nanoparticles may be attributed to the specific phytochemicals present in the plant extract, which may not interact as effectively with MTB as those from other plants (Ganjalikhani-Hakemi & Baghery, 2020). Additionally, the synthesis conditions and the resulting nanoparticle characteristics play a crucial role in determining their antimicrobial activity.

Cytotoxicity and Safety Considerations

The low cytotoxicity observed with Au-AgNPs derived from *Acalypha indica* suggests that these nanoparticles are promising candidates for further development as anti-TB agents. The safety profile of nanoparticles is a critical consideration in their therapeutic application, and the lack of significant cytotoxicity at effective concentrations enhances their suitability for in vivo studies and potential clinical use (Azam et al., 2020; Zhao & Huang, 2019). The moderate cytotoxicity observed with AgNPs and AuNPs at higher concentrations indicates that careful optimization of dosage is required to balance efficacy and safety. The cytotoxic effects of nanoparticles are often influenced by their size, shape, and surface characteristics, which can affect cellular uptake and interaction with host cells (Barabadi et al., 2018; McCarthy & Byrne, 2020). Thus, future research should focus on optimizing these parameters to minimize cytotoxicity while maximizing anti-mycobacterial activity (El-Sayed et al., 2005).

Implications for Future Research and Therapeutic Development

The findings of this study highlight the potential of bimetallic nanoparticles, particularly those derived from Acalypha indica, as viable candidates for anti-TB therapy. The enhanced anti-mycobacterial activity and low cytotoxicity make these nanoparticles suitable for further preclinical and clinical evaluations (Kesharwani et al., 2020). Future research should explore the detailed mechanisms of action of these nanoparticles, including their interaction with MTB at the molecular level, to better understand their therapeutic potential (Nithya & Sundrarajan, 2020).

The optimization of nanoparticle synthesis parameters and formulation strategies will be crucial in enhancing their efficacy and safety profile. Studies on the stability, pharmacokinetics, and bioavailability of these nanoparticles are essential to ensure their effective delivery and therapeutic outcomes (El-Batal et al., 2020; Kumar & Yadav, 2009). Exploring the synergistic effects of combining nanoparticles with existing anti-TB drugs may also provide new avenues for improving treatment regimens and overcoming drug resistance (Azam et al., 2020; Bhattacharya & Kundu, 2018).

Conclusion

The study demonstrates that bimetallic Au-Ag nanoparticles synthesized using Indian medicinal plants exhibit superior anti-tubercular activity compared to their mono-metallic counterparts. The enhanced efficacy of Au-AgNPs, particularly those derived from Acalypha indica, can be attributed to the synergistic effects of combining silver and gold, which improve interaction with Mycobacterium tuberculosis. This synergy enhances antimicrobial properties through a combination of oxidative stress and enhanced cellular uptake. While gold nanoparticles showed better activity than silver nanoparticles, the lower cytotoxicity of Au-AgNPs at effective concentrations suggests promising therapeutic potential. Future research should focus on optimizing synthesis parameters, exploring detailed mechanisms of action, and evaluating the stability and bioavailability of these nanoparticles to develop novel and effective anti-TB therapies. This approach aligns with the integration of traditional medicine and advanced nanotechnology.

Author contributions

G.R., conceptualized and developed the methodology, G.S., prepared the original draft and collected, E.M., reviewed and edited the writing.

Acknowledgment

The author thanks the Department of Microbiology, Govt Theni Medical College and Hospital, Theni, Tamil Nadu-625 512, India.

Competing financial interests

The authors have no conflict of interest.

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