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ANTIBACTERIAL EFFICACY OF SPHATIKA BHASMA AND BHASMA DERIVED SILVER NANOPARTICLES: A TRADITIONAL REMEDY IN A MODERN FORM

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ABSTRACT

The utilization of Ayurvedic Bhasma is well-established in managing chronic diseases, while the introduction of silver nanoparticles signifies a contemporary advancement with extensive applications in healthcare. This research study meticulously evaluated the antibacterial efficacy of Sphatika Bhasma and silver nanoparticles derived from Sphatika Bhasma against multidrug-resistant isolates of *Pseudomonas aeruginosa* (Gram-negative) and *Enterococcus faecalis* (Gram-positive) through a minimum inhibitory concentration (MIC) assay. Different concentrations—2.5, 5.0, and 10 mg for Bhasma and 2.5, 5.0, and 10 µg for silver nanoparticles were employed for this evaluation. Characterization of the Bhasma and AgNPs was performed using UV vis Spectroscopy and XRD analysis. The findings revealed that the Bhasma and silver nanoparticles exhibited significant antibacterial activity against the target isolates. Notably, Sphatika Bhasma showcased the highest zone of inhibition against *P. aeruginosa* at a concentration of 10 mg. In contrast, the highest level of growth inhibition for AgNPs was recorded against *E. faecalis* at a concentration of 10 µg. These results highlight the potential of Sphatika Bhasma and its nano-derivatives as antimicrobial agents and encourage further exploration of nanotechnology-based strategies to address the increasing challenge of antimicrobial resistance, particularly in bacterial infections.

INTRODUCTION

The global rise in antibiotic resistance demands innovative antimicrobial solutions and re-evaluating traditional medical practices (Ruttkay-Nedecky et al., 2018). Although medical progress has advanced significantly, infectious diseases continue to be a leading cause of mortality globally, emphasizing the ongoing necessity for developing novel approaches to prevention and therapy(Amini, 2019). Nanotechnology allows the manipulation of materials at the molecular level, creating opportunities for new antimicrobial agents and drug delivery systems. It holds potential for early disease detection, treatment, and prevention. Traditional medicine offers valuable insights, with various natural substances displaying antimicrobial properties that can give rise new therapies (Mody et al., 2010). Sphatika Bhasma, an Ayurvedic formulation made from alum, has been used for its antiseptic and wound-healing effects (Sanchez et al., 2021). Combining nanotechnology with traditional medicine can enhance the therapeutic effectiveness of substances like Sphatika Bhasma by synthesising nanoparticles (Gurunathan et al., 2014). Silver nanoparticles are particularly noteworthy for their strong antibacterial properties and effectiveness against multidrug-resistant bacteria, making them promising for hard-to-treat infections (Aljeldah et al., 2023). Their high surface area-to-volume ratio improves interactions with bacterial cells, resulting in better bactericidal effects than bulk materials (Ssekatawa et al., 2021). Integrating nanotechnology with traditional medicine can create more effective and less toxic antimicrobial agents, potentially transforming the treatment of infectious diseases. This approach builds on extensive existing research, indicating a growing interest in alternatives to conventional medications.

Sphatika Bhasma is prepared through a detailed process of purification and calcination, resulting in a fine powder with enhanced bioavailability and therapeutic efficacy. Traditionally, it is known for its astringent and cooling properties, which aid in wound healing and possess anti-inflammatory effects (Raju et al., 2020). The calcination, or Bhasma preparation, enhances its therapeutic potential while reducing toxicity. Used both internally and externally, Sphatika Bhasma addresses various conditions, including skin infections and bleeding disorders. Its historical application supports research into its potential for synthesizing antibacterial nanoparticles, merging traditional

knowledge with modern nanotechnology for innovative therapies (Fernando et al., 2018).
Silver nanoparticles are promising antimicrobial agents due to their ability to kill bacteria, viruses, and fungi (Tammam et al., 2021). They disrupt bacterial cell membranes, interfere with DNA replication, and produce reactive oxygen species (Mody et al.,2010).Their effectiveness depends on size, shape, and surface charge, with smaller particles generally being more effective (Mody et al., 2010). However, their safety is crucial as toxicity increases with size and relates to how they produce reactive oxygen species and release silver ions. Studies show that silver nanoparticles are effective against drug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococci*, demonstrating their potential to combat antibiotic resistance. Developing silver nanoparticle-based antimicrobial agents may improve treatment options for infections in light of rising antibiotic resistance (Kannan et al., 2017).

The AgNPs synthesis from Sphatika Bhasma merges traditional medicine with nanotechnology, enhancing its antibacterial properties. This eco-friendly method reduces toxic solvent use and environmental impact. The resulting nanoparticles have unique properties that boost their antimicrobial activity (Ahmad et al., 2011). By combining traditional knowledge and modern technology, we can develop effective agents to combat antibiotic resistance. Nanomaterials work against resistance by disrupting cell membranes, generating reactive oxygen species, and eliminating biofilms (Chen et al., 2020). This investigation aimed to evaluate the antimicrobial efficacy of Sphatika Bhasma and its synthesised silver nanoparticles against multidrug-resistant bacterial strains, to explore their potential as alternative therapeutic agents that merge traditional Ayurvedic medicine with modern nanotechnology.

Materials and Methodology

Isolation of Bacteria

In this study, five isolates of*Enterococcus faecalis* and*Pseudomonas aeruginosa* were isolated from clinical samples and cultured on UTI Agar plates, specifically designed to support the growth of urinary pathogens. These isolates were meticulously processed to ensure purity and viability. Following isolation, comprehensive antibiotic sensitivity testing was conducted, utilising a range of antibiotics to evaluate the antibiotic sensitivity pattern of these clinically relevant bacteria.

Antibacterial Activity of Sphatika Bhasma

The antibacterial potential of Sphatika Bhasma was tested using the well diffusion method. Solutions at concentrations of 2.5, 5.0, and 10 mg were prepared in distilled water and loaded into wells created in an agar medium inoculated with specific bacterial isolates. After incubation, the inhibition zones were measured to evaluate the antibacterial potential of Sphatika Bhasma at the various concentrations.

Synthesis of Silver Nanoparticles

A 1 mM $AgNO_3$ solution (Hi Media) was prepared to synthesise silver nanoparticles, with Spahtika Bhasma suspensions at 1 mg/ml prepared by vortexing. The solutions were mixed in a 9:1 ratio and allowed to react undisturbed for 24 hours in a dark chamber to minimise photo-activation. Colour change and plasmon resonance were observed to confirm nanoparticle formation. The suspension was centrifuged at 10000 rpm for 2 hours, the supernatant was discarded, the pellet subjected washed with distilled water before drying for further analysis.

XRD Analysis

The characterization of Sphatika Bhasma and Sphatika Bhasma AgNPs was conducted using X-ray Diffraction. This method helps to evaluatethe samples' structural properties.

Antibacterial Activity of Sphatika Bhasma Silver Nanoparticles

The synthesised silver nanoparticles (AgNPs) of Bhasma were tested for their antibacterial efficacy. A well-diffusion method was used to evaluate their antibacterial activity. The effects of these nanoparticles were tested at various concentrations of 2.5, 5.0, and 10 µg to determine their efficacy against specific bacterial isolates.

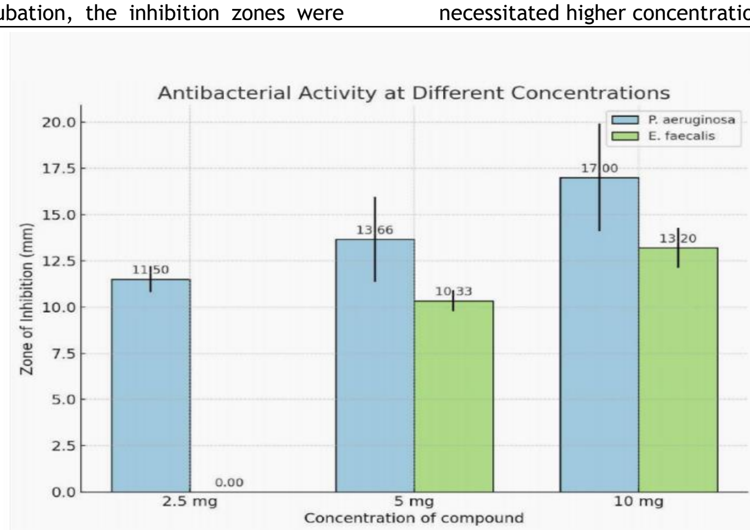
RESULT

Isolation of Bacteria

On UTI agar, the distinct colony morphologies helped preliminary identification of bacterial species. *Pseudomonas aeruginosa* colonies were large and mucoid, showing a characteristic greenish-blue pigmentation. In contrast, *Enterococcus faecalis* colonies were small and discrete, displaying a uniform green colouration. These differential characteristics enabled the initial differentiation of the organisms based on their cultural characteristics observed on the selective medium.

Antibacterial Activity of Sphatika Bhasma

The effectiveness of Sphatika Bhasma in inhibiting the growth of *Enterococcus faecalis* and *Pseudomonas aeruginosa* was tested by well diffusion assay; the size of the inhibition zones at different concentrations of 2.5 mg, 5 mg, and 10 mg was measured. The results demonstrated a dose-dependent response in *P. aeruginosa*, with a statistically significant increase in the zone of inhibition correlated with higher concentrations of the test compound (Graph 1). In contrast, *E. faecalis* exhibited a lower susceptibility to the test compound, with no inhibition observed at a concentration of 2.5 mg (0.00 ± 0.00 mm). At higher concentrations, measurable inhibition zones were detected (Graph 1). These findings indicate that the test compound exhibits a significantly more substantial and consistent antibacterial effect against *P. aeruginosa* than *E. faecalis*, which demonstrated reduced sensitivity and necessitated higher concentrations for an inhibitory response.



Graph 1 - Antibacterial Activity of Sphatika Bhasma

Synthesis of Silver Nanoparticles-
The preliminary confirmation of silver nanoparticles synthesized from Sphatika Bhasma was performed through visual observations and surface plasmon resonance analysis. A noticeable change in the colour of the solution was observed, shifting from a slightly milky white to a brown-red colour, indicating the formation of silver nanoparticles. Furthermore, UV-Vis spectroscopy showed that the SPR peak shifted to 341.2 and 500 nm nm after 24 hours, confirming the presence of the silver nanoparticles.

XRD Analysis-
The crystalline structure of synthesized AgNPsusing Sphatika Bhasma was validated through X-ray diffraction (XRD) analysis. The resulting XRD pattern (Figure 1) exhibited distinct and sharp diffraction peaks at 2θ angles of approximately 38°, 44°, 64°, and 77°, which correspond to the (111), (200), (220), and (311) crystallographic planes of silver, respectively. These observations are in line with the data provided in JCPDS file No. 04-0783, confirming the formation of metallic silver in a crystalline phase. The pronounced intensity and definition of the observed peaks suggest a morecrystalline nature among the nanoparticles. The minor broadening of peaks, particularly at lower angles, is indicative of the nanoscale dimensions of the particles. Additionally, no extraneous peaks associated with impurities were detected, thereby confirming the phase purity of the AgNPssynthesized.

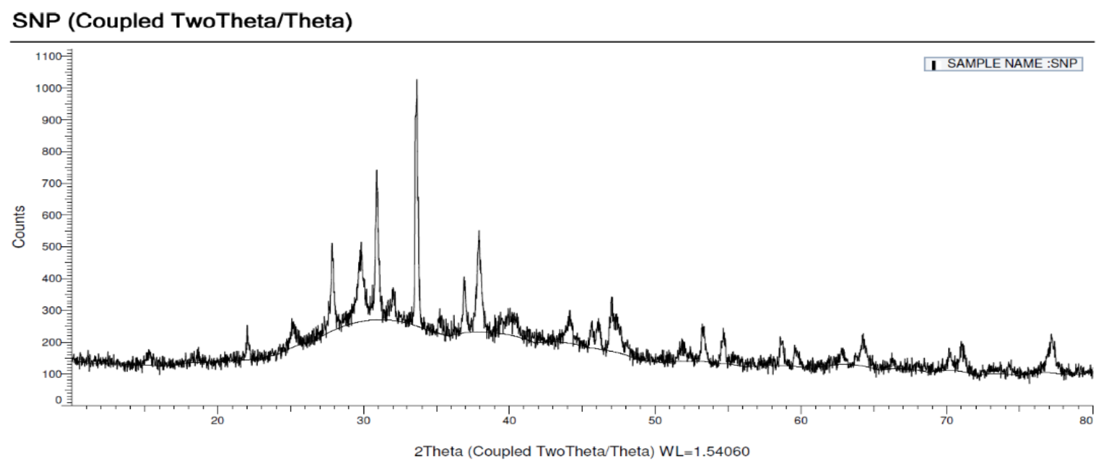
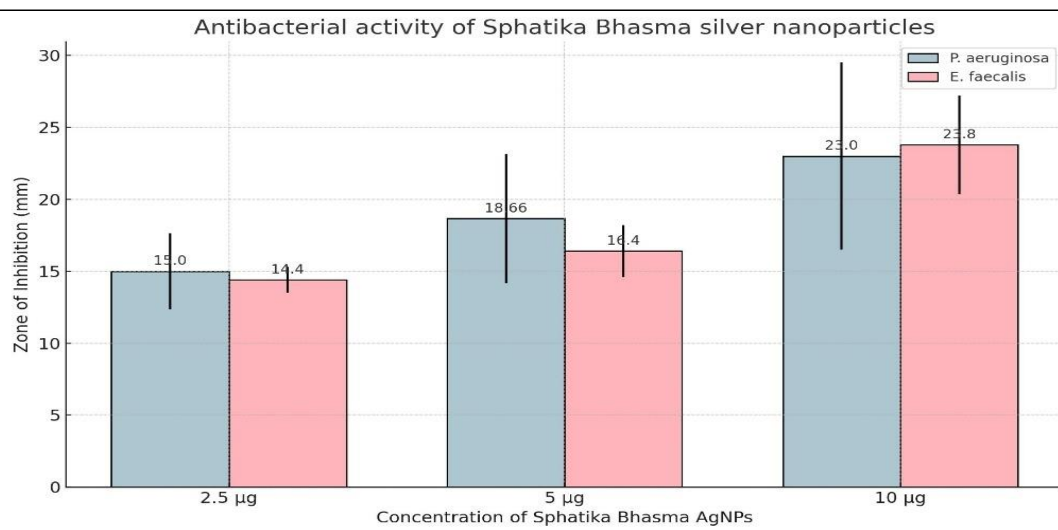


Figure 1- XRD of Sphatika Bhasma Silver Nanoparticles

Antibacterial Activity of Sphatika Bhasma Silver Nanoparticles
The antibacterial activity of Sphatika Bhasma silver nanoparticles showed an apparent increase in effectiveness depending on the concentration against both*Enterococcus faecalis*and*Pseudomonas aeruginosa* (Graph 2). The resultsindicated that the nanoparticles worked well against both organisms, with *E. faecalis* being slightly more susceptible at the highest concentration. These findings suggest that Sphatika Bhasma AgNPs could serve as a broad-spectrum antibacterial agent, with enhanced effectiveness at higher doses.



Graph- 2:Antibacterial Activity of Sphatika Bhasma Silver Nanoparticles

DISCUSSION

The current study demonstrates the promising antibacterial potential of Sphatika Bhasma and Bhasma-derived silver nanoparticles against both *Enterococcus faecalis*and*Pseudomonas aeruginosa*, supported by their concentration-dependent activity. Notably, *P. aeruginosa* showcased consistent susceptibility across both Sphatika Bhasma and its nanoparticle form, while *E.*

faecalis displayed significant inhibition only at higher concentrations. The enhanced antibacterial potential of the nanoparticle formulation compared to the Bhasma suggests that nanosizing increases the surface area and reactivity, improving microbial interaction and penetration (Rai et al., 2009; Kim et al., 2007).

The observed differences in susceptibility of the two bacterial strains may be attributed to structural variations. *P. aeruginosa*, a Gram-negative bacterium, possesses a thinner peptidoglycan layer and an outer membrane rich in porins, which may allow better uptake of AgNPs (Morones et al., 2005). Conversely, *E. faecalis*, a Gram-positive organism, has a thicker peptidoglycan wall that may initially limit nanoparticle penetration. However, its susceptibility at higher concentrations indicates that the particles can overcome this barrier when present in sufficient amounts (Liao et al., 2019). Interestingly, at the highest tested concentration (10 µg), *E. faecalis* exhibited a slightly larger zone of inhibition than *P. aeruginosa*, which could be due to strain-specific sensitivity or differences in nanoparticle-bacteria surface interactions.

The X-ray diffraction study analysis further substantiates the formation and crystalline nature of the silver nanoparticles synthesized using Sphatika Bhasma. The diffraction peaks observed at 2θ values of approximately 38°, 44°, 64°, and 77° correspond well with the face-centered cubic (FCC) structure of elemental silver, as reported in JCPDS No. 04-0783. The absence of extraneous peaks suggests high phase purity, while the sharpness and intensity of the peaks indicate well-defined crystalline domains (Song and Kim, 2009). Minor broadening at lower angles is characteristic of nanoscale crystallites and is consistent with previous reports on AgNPs synthesized using green or mineral-based methods (Ahmed et al., 2016).

The integration of traditional Ayurvedic preparation with nanotechnology, as exemplified by Sphatika Bhasma AgNPs, offers a novel and potentially scalable approach to developing broad-spectrum antibacterial agents. These findings are especially significant in the context of increasing antibiotic resistance, as silver-based nanomaterials have shown efficacy against drug-resistant strains through multifaceted mechanisms such as membrane disruption, protein inactivation, and ROS generation (Franci et al., 2015). Further studies are warranted to explore the mechanistic pathways, cytotoxicity, and in vivo efficacy of these formulations.

CONCLUSION

In conclusion, the study demonstrates the significant antibacterial potential of Sphatika Bhasma and silver nanoparticles derived from Sphatika Bhasma, showing effective, dose-dependent inhibition against *Pseudomonas aeruginosa* and *Enterococcus faecalis*. The crystalline structure and purity of the synthesized AgNps, confirmed by X-ray diffraction analysis, highlight the successful preparation of these materials. The superior antibacterial efficacy of the nanoparticle formulation compared to Bhasma underscores the benefits of nanoscale processing in enhancing antimicrobial action. These results not only support the validity of traditional Ayurvedic preparations using modern scientific techniques but also indicate that Sphatika Bhasma-based silver nanoparticles hold promise as broad-spectrum antibacterial agents in the face of rising antibiotic resistance. Further in vivo studies and toxicity profiling to fully evaluate their therapeutic potential is recommended.

REFERENCES

Ahmad, N., Sharma, S., Singh, V. N., Shamsi, S. F., Fatma, A., & Mehta, B. R. (2011). Biosynthesis of silver nanoparticles from Desmodiumtriflorum: a novel approach towards weed utilization. Biotechnology research international, 2011(1), 454090.

Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2016). Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. *Journal of radiation research and applied sciences*, *9*(1), 1-7.

Aljeldah, M. M., Yassin, M. T., Mostafa, A. A. F., & Aboul-Soud, M. A. (2023). Synergistic antibacterial potential of greenly synthesized silver nanoparticles with fosfomycin against some nosocomial bacterial pathogens. *Infection and Drug Resistance*, 125-142.

Amini, S. M. (2019). Preparation of antimicrobial metallic nanoparticles with bioactive compounds. *Materials Science and Engineering: C*, *103*, 109809.

Chen, Y., Ren, L., Sun, L., Bai, X., Zhuang, G., Cao, B., ... & Liu, S. (2020). Amphiphilic silver nanoclusters show active nano–bio interaction with compelling antibacterial activity against multidrug-resistant bacteria. NPG Asia Materials, 12(1), 56.

Fernando, S. S. N., Gunasekara, T. D. C. P., & Holton, J. (2018). Antimicrobial nanoparticles: applications and mechanisms of action.

Franci, G., Falanga, A., Galdiero, S., Palomba, L., Rai, M., Morelli, G., &Galdiero, M. (2015). Silver nanoparticles as potential antibacterial agents. *Molecules*, *20*(5), 8856-8874.

Gurunathan, S., Han, J. W., Kwon, D. N., & Kim, J. H. (2014). Enhanced antibacterial and anti-biofilm activities of silver nanoparticles against Gram-negative and Gram-positive bacteria. *Nanoscale research letters*, *9*, 1-17.

Kannan, M., Rajarathinam, K., Venkatesan, S., Dheeba, B., & Maniraj, A. (2017). Silver Iodide Nanoparticles as an Antibiofilm Agent—A Case Study on Gram-Negative Biofilm-Forming Bacteria. In *Nanostructures for antimicrobial therapy* (pp. 435-456).

Kim, J. S., Kuk, E., Yu, K. N., Kim, J. H., Park, S. J., Lee, H. J., ... & Cho, M. H. (2007). Antimicrobial effects of silver nanoparticles. *Nanomedicine: Nanotechnology, biology and medicine*, *3*(1), 95-101.

Liao, C., Li, Y., & Tjong, S. C. (2019). Bactericidal and cytotoxic properties of silver nanoparticles. *International journal of molecular sciences*, *20*(2), 449.

Mody, V. V., Siwale, R., Singh, A., & Mody, H. R. (2010). Introduction to metallic nanoparticles. *Journal of Pharmacy and bioallied sciences*, *2*(4), 282-289.

Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramírez, J. T., &Yacaman, M. J. (2005). The bactericidal effect of silver nanoparticles. *Nanotechnology*, *16*(10), 2346.

Rai, M., Yadav, A., & Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnology advances*, *27*(1), 76-83.

Raju, S., Ashok, D., & Vinuthna, C. (2020). Comparative Antimicrobial Activity of Phytofabricated Ag and Au Nanoparticles from Ledebouriahydrabadensis Rhizome using Various Methods. *Indian Journal of Pharmaceutical Sciences*, *82*(5).

Ruttkay-Nedecky, B., Skalickova, S., Kepinska, M., Cihalova, K., Docekalova, M., Stankova, M., ... & Kizek, R. (2019). Development of new silver nanoparticles suitable for materials with antimicrobial properties. *Journal of nanoscience and nanotechnology*, *19*(5), 2762-2769.

Sanchez, S. V., Navarro, N., Catalán-Figueroa, J., & Morales, J. O. (2021). Nanoparticles as potential novel therapies for urinary tract infections. *Frontiers in cellular and infection microbiology*, *11*, 656496.

Song, J. Y., & Kim, B. S. (2009). Rapid biological synthesis of silver nanoparticles using plant leaf extracts. *Bioprocess and biosystems engineering*, *32*, 79-84.

Ssekatawa, K., Byarugaba, D. K., Kato, C. D., Wampande, E. M., Ejobi, F., Nakavuma, J. L., ... & Kirabira, J. B. (2021). Green strategy–based synthesis of silver nanoparticles for antibacterial applications. *Frontiers in Nanotechnology*, *3*, 697303.

Tammam, A. M., Ibrahim, S. A., Hemid, A. A., Abdel-Azeem, F., El-Faham, A. I., Ali, N. G., & Salem, W. (2021). Effect of silver nanoparticles as a water supplementation on productive performance, carcass characteristics and bone measurements of broiler chicks. *Egyptian Journal of Nutrition and Feeds*, *24*(2), 95-100.