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Green Synthesis and Characterization of Silver Nanoparticles Using *Stachytarpheta indica*

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A B S T R A C T

The present study explores the green synthesis of silver nanoparticles (AgNPs) using *Stachytarpheta indica* extract as a reducing and stabilizing agent. The synthesized nanoparticles were characterized using UV-Vis spectrophotometry, Fourier-transform infrared (FTIR) spectroscopy, and scanning electron microscopy (SEM). UV-Vis analysis confirmed the successful formation of AgNPs with a characteristic surface plasmon resonance (SPR) peak at 420 nm, indicative of well-dispersed and stable nanoparticles. FTIR spectroscopy identified functional groups, such as hydroxyl (-OH) and carbonyl (C=O), involved in nanoparticle stabilization. SEM imaging revealed distinctive floral-shaped nanostructures with an approximate size of 11.5 µm at 1000X magnification, suggesting effective bio-reduction by phytochemicals. These findings align with previous studies on plant-mediated AgNP synthesis, reinforcing the potential of *S. indica* for eco-friendly nanotechnology applications. The synthesized AgNPs exhibit promising characteristics for antimicrobial and biomedical applications, highlighting their significance in sustainable nanoscience.

Introduction

Nanoparticles, often ranging from 1 to 100 nanometers in size, exhibit unique properties that differ from their bulk material counterparts due to their small size, large surface area-to-volume ratio, and quantum effects. These properties make them ideal candidates for biomedical applications, especially in drug delivery, where precision and controlled releases are crucial. The use of nanoparticles can improve drug solubility, stability, and bioavailability, leading to more effective treatments with reduced side effects (Zhang *et al.,* 2020). Green synthesis of nanoparticles is an environmentally friendly approach that utilizes plant extracts (phytoextracts) to produce nanoparticles with enhanced bioactivity. Unlike conventional chemical and physical methods of nanoparticle synthesis, which often involve toxic reagents and harsh conditions, green synthesis offers a sustainable alternative. Phytoextracts contain natural biomolecules such as alkaloids, flavonoids, terpenoids, and phenolic compounds that act as reducing and stabilizing agents during nanoparticle formation (Sharma *et al.,* 2019). This method not only reduces the need for hazardous chemicals but also provides nanoparticles with biocompatibility and enhanced antimicrobial, antioxidant, and anti-inflammatory properties (Iravani, 2011).

The environmental benefits of green synthesis are significant. It minimizes the production of toxic by-products and the use of non-renewable resources, contributing to the reduction of environmental pollution. Additionally, green synthesis typically operates under ambient temperature and pressure, reducing the energy consumption associated with conventional methods (Ahmed *et al.,* 2016). By relying on naturally occurring compounds in plants, green synthesis also supports biodiversity conservation by utilizing abundant and renewable resources.

Another major advantage of green-synthesized nanoparticles is their potential for enhanced bioactivity. Studies have shown that nanoparticles synthesized using phytoextracts exhibit improved antimicrobial and anticancer properties compared to those produced via traditional methods (Patra & Baek, 2014). The synergistic effects of bioactive phytochemicals with the nanoparticles confer additional biological activities, making these nanoparticles more effective in therapeutic applications. For example, silver nanoparticles synthesized using *Stachytarpheta indica* extracts have demonstrated significant antimicrobial effects, highlighting their potential in combating drug-resistant pathogens (Sathiyavimal *et al.,* 2020).

Green synthesis of nanoparticles using plant extracts represents a promising approach to developing biocompatible and environmentally friendly nanomaterials with enhanced bioactivity for various biomedical applications.

Nanoparticles, particularly metal nanoparticles (zinc, copper, silver, gold, magnesium, titanium) are being applied in various fields due to their distinctive properties. They have properties totally different from the bulk metal from which they are formed. Their application includes molecular imaging, catalysis, water treatment, diagnosis, drug delivery, wound healing, cosmetics, clothing, sunscreens, food industry, etc. They also keep properties like antimicrobial, antiviral, antidandruff, anti-helmintic, anti-inflammatory, analgesic, anticoagulant, antidiabetic, antioxidant, anticancer, anti-proliferative activities and also show antigenotoxic, cytotoxic effect (Chaudhuri and Paria, 2012; Govindappa *et al.,* 2018).

Using plant extracts, synthesis of metal nanoparticles is simple, easy and ecofriendly. Usually, when plant extracts are allowed to react with the particular metal salt, leads to the reduction of metal by plant secondary metabolites into metal nanoparticles; where secondary metabolites in plant extract acts as reducing agent as well as stabilizing agents. Any plant part *viz*., leaf, stem, flower, fruit, seed, root, bark, etc. can be used for the synthesis of nanoparticles (Murali *et al.,* 2021; Bharadwaj *et al.,* 2021; Khan *et al.,* 2022).

However, despite these promising developments, the use of nanotechnology in medicine also raises certain challenges. Issues related to biocompatibility, long-term toxicity, and the immune response to nanoparticles remaining areas of concern. More research is needed to ensure the safe and effective translation of these technologies from laboratory settings to clinical applications (Salvati *et al.,* 2013).

Materials and Methods

Synthesis of Phyto-Extract Mediated Nanoparticles

Silver nanoparticles were synthesized following a modified green synthesis protocol. Aqueous extracts of Stachytarpheta indica leaves were prepared and used as reducing and stabilizing agents. A 0.1 M silver nitrate ($AgNO_3$) solution was prepared, and 5 mL of *S. indica* extract was added dropwise under continuous stirring at 40°C. The color change from transparent to reddish-brown indicated AgNP formation. The solution was incubated for 6 hours and centrifuged at 5000 rpm for 10 minutes. The obtained nanoparticles were dried at 45°C and stored for further analysis.

For present investigation nanoparticle will be prepared by treating the solution of salt of silver & zinc with phytochemical extracts of plant opted in present study would be prepared according to the method suggested by Sharma *et al.,* (2018); Stan *et al.,* (2016); Venkateasan *et al.,* (2017) and Happy *et al.,* (2019) with suitable modifications.

Silver nitrate and Zinc sulphate solution will be used as precursor to treat with phytochemical extract to form phytoextract mediated AgNPs and ZnNPs. The nanoparticles will be prepared by reducing the precursor solutions by phytochemical extract of plant material.

The formation of nanoparticle would be monitored by observing the change in colour and formation of sediments at suitable temperature & pH.

Synthesis of Silver Nanoparticle with Extract

For synthesis of silver nanoparticle, a known concentration of leaf extract was allowed to interact with 0.1 M $AgNO_3$ solution at a definite mixing ratio to make up 100 ml volume in 250 ml conical flasks.

For this purpose, the 45 ml of 0.1 M aqueous solution $AgNO_3$ was prepared. The transparency and clarity of the solution was checked. To this solution 5 ml of *Stachytarpheta indica* L. aqueous extract from 100 mg/ml stock was added. The adding of phytochemical extract was maintained drop wise into the 0.1 M $AgNO_3$ aqueous solution with continuous stirring at magnetic stirrer at 40ºC temperature.

Aqueous Extraction of Crud Drug

About 50 gram of fine leaf powder of *Stachytarpheta indica* L. was subjected to defatted in petroleum ether first by keeping the powder dipped in overnight.

After defatting the leaf powered was allowed to further drying at room temperature by spreading it on newspaper leaving for 3-4 hours leads to complete evaporation of petroleum ether.

Now 20 grams of defatted dried powder was subjected to Soxhlet extraction with 200 ml of distilled water as a solvent. Soxhlation process was allowed to carry out for 24 hours at 70ºC temperature till the exhaustion of the merc (Handa, 2008).

The extract so obtained is subjected to evaporation of solvent in boiling water bath

$$\text{Percentage Yield} = \frac{\text{Weight of Extract Drug}}{\text{Weight of Crude Drug}} \times 100$$

At continuous stirring condition change in colour from transparent to yellow to redish brown within hour indicates the formation of Silver Nano Particles of plant extract (Valli *et al.,* 2012). The stirring was maintained for 5 to 6 hours, after which the solution was allowed to stand for overnight. This was followed by separation of AgNPs by centrifugation at 5000 rpm at room temperature for 10 min they drying of pellets was done at 45°C for 24 h and the powder so obtained was dispensed in sterile, distilled water to obtain the required experimental concentration for the experiments.

Characterization Techniques

UV-Vis Spectrophotometry: The optical properties of AgNPs were analyzed using a UV-Vis spectrophotometer, with absorbance recorded between 300–800 nm.

Fourier-Transform Infrared (FTIR) Spectroscopy: FTIR analysis identified functional groups responsible for nanoparticle reduction and stabilization.

Scanning Electron Microscopy (SEM): SEM imaging provided insights into the morphology, size distribution, and surface characteristics of the synthesized AgNPs.

Results and Discussion

Analysis of Phyto-Extract Mediated Nanoparticles

The characterization of silver and zinc nanoparticles synthesized using *Stachytarpheta indica* extract was performed through UV-Vis spectrophotometry, FTIR analysis, and SEM imaging. These techniques provided crucial insights into the formation, functional group interactions, and morphological attributes of the nanoparticles. The UV-Vis spectrophotometry confirmed the successful synthesis by detecting characteristic surface plasmon resonance peaks.

FTIR analysis identified functional groups responsible for nanoparticle stabilization, indicating the involvement of bioactive compounds from the extract. SEM imaging revealed the morphological structure, size distribution, and surface characteristics of the synthesized nanoparticles. The outcomes of these analyses are discussed in detail in the following sections.

Analysis of Silver Nanoparticle

UV-Vis Spectrophotometry

The formation of silver nanoparticles was confirmed by UV-Vis spectrophotometric analysis, which displayed a distinct surface plasmon resonance (SPR) peak within the expected range, indicating stable nanoparticle synthesis. UV-Vis absorbance spectrum of *Stachytarpheta indica* aqueous extract and their respective silver nanoparticles is depicted in figure 1. The UV-Vis spectrophotometry analysis of *Stachytarpheta indica*-mediated silver nanoparticles (SIAgNP) exhibited a characteristic surface plasmon resonance (SPR) peak at 420 nm with a maximum absorbance of 3.6, confirming the successful synthesis of silver nanoparticles. Peaks generated after scanning the analyte in range from 300 nm to 800 nm on UV-Vis spectrophotometer LabIndia3000+. This peak falls within the typical SPR range (400–450 nm) for silver nanoparticles, indicating the presence of well-dispersed and stable nanostructures. In contrast, the aqueous extract of *Stachytarpheta indica* (SI Aq Extract) showed a broader absorbance spectrum without a distinct peak, suggesting the presence of various phytochemicals responsible for nanoparticle reduction and stabilization. The decline in absorbance at higher wavelengths for SIAgNP indicates reduced aggregation and stable colloidal dispersion.

These findings confirm the formation of silver nanoparticles with characteristic optical properties, essential for their further antimicrobial evaluation.

The UV-Vis spectrophotometric analysis of silver nanoparticles (AgNPs) synthesized using *Stachytarpheta indica* extract revealed a distinct surface plasmon resonance (SPR) peak at 420 nm, indicative of successful nanoparticle formation. This observation aligns with previous studies employing plant extracts for AgNP synthesis. For instance, AgNPs synthesized using *Coptidis rhizome* extract exhibited an SPR peak at 420 nm, confirming nanoparticle formation (Kim *et al.,* 2007). Similarly, AgNPs produced with *Pedalium murex* leaf extract showed an SPR peak around 430 nm, consistent with the characteristic SPR range for AgNPs (Elumalai *et al.,* 2015). These consistent SPR peaks across different plant-mediated syntheses underscore the efficacy of phytochemicals in reducing and stabilizing silver ions into nanoparticles. The uniformity in SPR peaks suggests that plant extracts can effectively control nanoparticle size and dispersion, crucial for applications in antimicrobial therapies.

FTIR Analysis

The FTIR spectrum of *Stachytarpheta indica* extract-mediated silver nanoparticles (AgNPs) as depicted in figure 4.4 was generated from FTIR instrument Alpha Bruker reveals key functional groups responsible for nanoparticle stabilization and reduction.

- Broad peak around 3300–3400 cm^{-1}: This corresponds to the stretching vibrations of hydroxyl (-OH) groups from phenolic compounds or flavonoids, which play a crucial role in reducing and capping the nanoparticles.
- Peaks around 2900 cm^{-1}: These are attributed to C-H stretching vibrations of alkanes, indicating the presence of organic biomolecules.
- Sharp peak around 1630 cm^{-1}: This represents C=O stretching vibrations from carboxyl (-COO) or amide groups, suggesting proteins or polyphenols involved in AgNP stabilization.
- Peaks between 1300–1400 cm^{-1}: These correspond to C-N and C-O stretching, indicative of amines, esters, or polysaccharides.
- Fingerprint region (below 1000 cm^{-1}): This signifies the presence of biomolecules interacting with silver ions.

Overall, the FTIR spectrum confirms the role of phytochemicals in *Stachytarpheta indica* extract as reducing and capping agents for silver nanoparticle synthesis.

Fourier-transform infrared (FTIR) spectroscopy is an essential technique for identifying biomolecules involved in the reduction, capping, and stabilization of silver nanoparticles (AgNPs). The FTIR spectrum of *Stachytarpheta indica*-mediated AgNPs (Figure 2.) revealed prominent peaks corresponding to various functional groups. The broad absorption peak near 3400 cm^{-1} indicates the presence of O-H stretching from phenolic and hydroxyl groups, which are known to act as reducing and stabilizing agents in nanoparticle synthesis (Iravani *et al.,* 2014). The peaks around 1630 cm^{-1} correspond to C=O stretching of amides or carboxyl groups, suggesting the involvement of proteins or flavonoids in nanoparticle capping.

Additionally, peaks in the region of 1000–1200 cm^{-1} confirm C-O stretching vibrations of polysaccharides, which could further aid in nanoparticle stability (Kalpana *et al.,* 2016).

Previous studies have reported similar findings where plant phytochemicals played a crucial role in AgNP formation. For example, Das *et al.,* (2020) demonstrated that flavonoids and terpenoids in *Azadirachta indica* extract acted as reducing and capping agents for AgNPs, enhancing their stability and bioactivity. The presence of these functional groups confirms the effective bioreduction of silver ions and suggests potential antimicrobial efficacy of the synthesized nanoparticles.

SEM Imaging

The SEM micrograph of *Stachytarpheta indica* extract-mediated silver nanoparticles (SIAgNP) as depicted in figure 3. captured at 1000X magnification provides key insights into the morphological characteristics of the synthesized nanoparticles which are as follows;

Particle Distribution and Morphology

The image shows a relatively uniform distribution of nanoparticles across the surface, suggesting effective stabilization of AgNPs by biomolecules present in the *Stachytarpheta indica* extract. The floral-shaped nanoparticles, as described, indicate possible hierarchical self-assembly, a common occurrence in green-synthesized nanoparticles due to organic capping agents such as flavonoids and phenolics.

Size and Structural Features

The scale bar (10 µm) and the stated size range (11.5 µm) indicate that the observed structures are in the micro to nanometer range. The presence of bright and darker regions suggests variations in surface topography, which could be due to the aggregation of nanoparticles or the interaction between biomolecules and silver ions during synthesis.

Surface Characteristics and Aggregation

The high-contrast regions in the SEM image suggest the presence of metallic silver clusters, with possible agglomeration of nanoparticles. Such formations can be influenced by reaction parameters, including pH, temperature, and extract concentration, as seen in earlier studies on biosynthesized AgNPs.

Overall, the SEM micrograph confirms the successful synthesis of silver nanoparticles with distinctive floral-shaped morphology, indicating the potential role of *S. indica* phytochemicals in the reduction and stabilization of AgNPs.

The SEM micrograph of *Stachytarpheta indica* extract-mediated silver nanoparticles (SIAgNPs) reveals floral-shaped nanostructures with a size range of approximately 11.5 µm at 1000X magnification (Figure 3). Previous studies have demonstrated that the morphology and size of biosynthesized nanoparticles are significantly influenced by the biomolecules present in plant extracts. For instance, Singh *et al.,* (2021) reported that flavonoids, phenolics, and terpenoids in plant extracts act as reducing and stabilizing agents, contributing to unique nanoparticle shapes, including spherical, rod-like, and flower-like morphologies. Similarly, Rajeshkumar *et al.,* (2019) observed that green-synthesized AgNPs using *Azadirachta indica* extract exhibited floral and dendritic structures, supporting the idea that plant metabolites play a crucial role in nanoparticle formation.

Furthermore, studies by Bar *et al.,* (2020) highlighted that nanoparticle aggregation and clustering are common in biosynthesis due to the interaction between silver ions and phytoconstituents, which explains the observed surface characteristics in the SEM image. The presence of distinct nanostructures enhances the antimicrobial and catalytic properties of AgNPs (Krishnaraj *et al.,* 2010). Therefore, the findings align with previous reports, reinforcing the potential of *S. indica* in green nanotechnology applications. The present study successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) using *Stachytarpheta indica* extract, highlighting an eco-friendly and sustainable approach. The phytochemicals in the extract acted as natural reducing and stabilizing agents, leading to the formation of well-dispersed nanoparticles.

UV-Vis spectrophotometry confirmed the presence of a characteristic surface plasmon resonance (SPR) peak at 420 nm, indicating successful nanoparticle synthesis. FTIR analysis identified key functional groups responsible for reduction and stabilization, while SEM imaging revealed distinct floral-shaped nanostructures with a size range of approximately 11.5 µm at 1000X magnification.

Figure.1 UV-Vis Absorbance Spectrum of Stachytarpheta indica Aqueous Extract and Silver Nanoparticles generated on UV-Vis spectrophotometer LabIndia3000+.

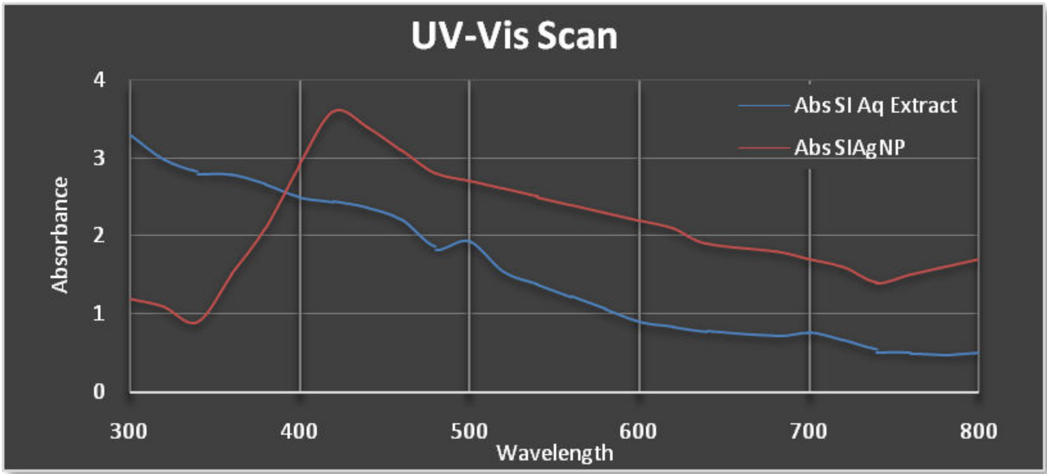


Figure.2 FTIR Spectrum of *Stachytarpheta indica* Extract-Mediated Silver Nanoparticles Showing Functional Groups Involved in Synthesis and Stabilization.

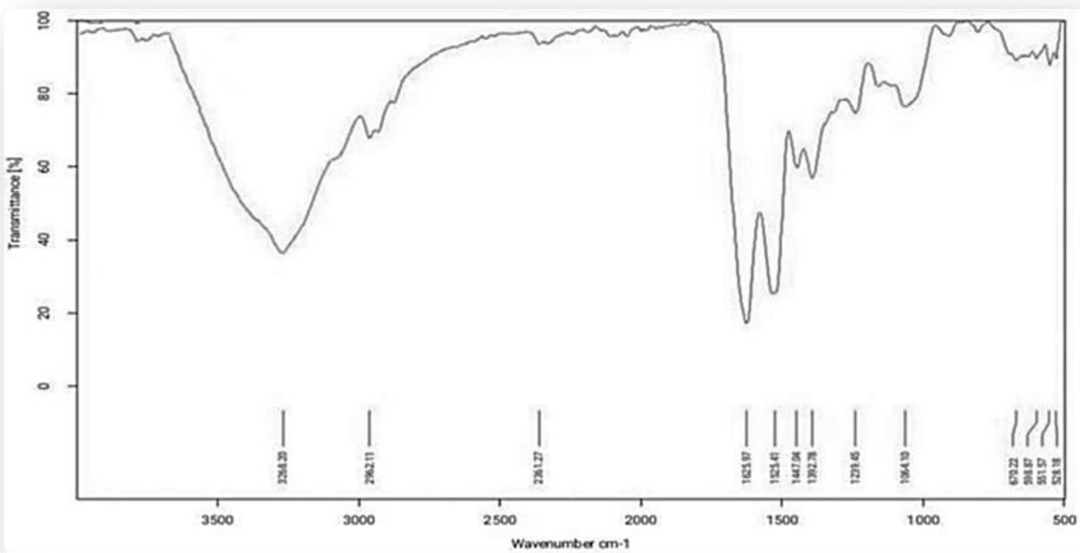
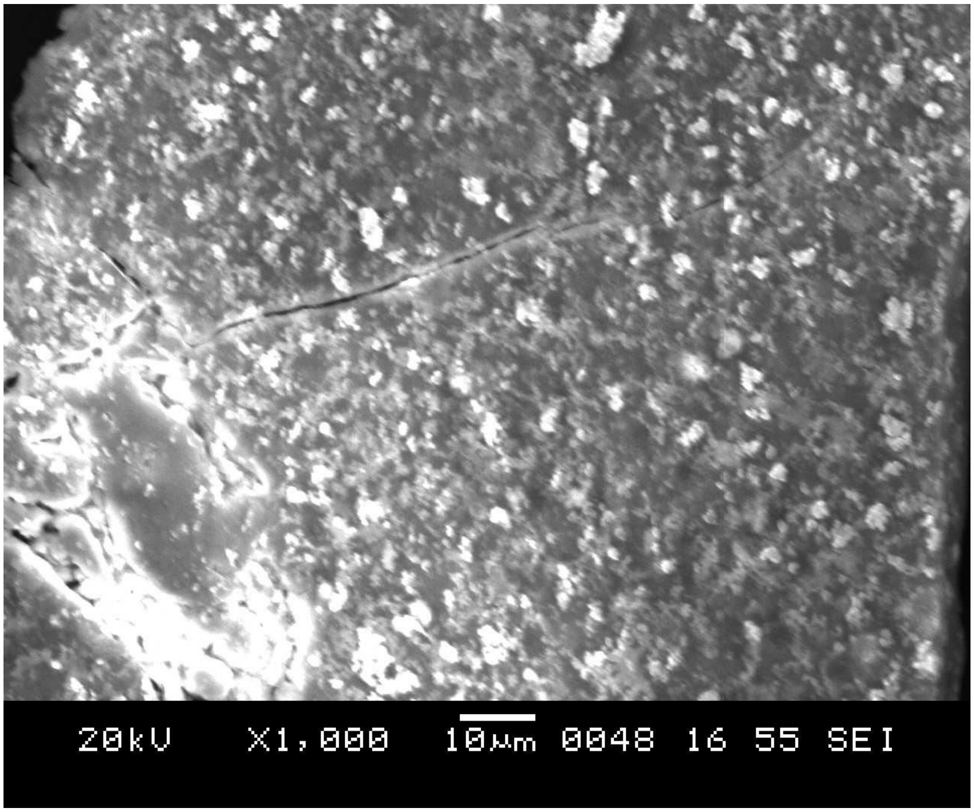


Figure.3 SEM micrograph of sample SIAgNP a *Stachytarpheta indica* extract-mediated silver nanoparticles captured at 1000X magnification.



The findings align with previous studies on plant-mediated AgNP synthesis, reinforcing the role of bioactive phytochemicals in controlling nanoparticle morphology and stability. The synthesized AgNPs exhibit promising potential for biomedical and antimicrobial applications due to their stability and unique structural characteristics. This green synthesis approach offers a cost-effective and environmentally friendly alternative to conventional nanoparticle synthesis methods, paving the way for further research into their potential therapeutic and industrial applications.

Author Contributions

Piyush Joshi: Investigation, formal analysis, writing—original draft. Laxmi kant Pandey: Validation, methodology, writing—reviewing. Vaibhav Misra:—Formal analysis, writing—review and editing. Ajad Patel: Investigation, writing—reviewing. Ranjan Singh: Resources, investigation writing—reviewing. Zareen Baksh: Validation, formal analysis, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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