Phyto-assisted synthesis of Silver nanoparticles using *Tinospora cordifolia* leaf extract and their antibacterial activity: An ecofriendly approach

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Abstract

To meet the increasing demands for commercial nanoparticles new eco-friendly methods of synthesis are being discovered. Plant mediated synthesis of nanoparticles offers single step, easy extracellular synthesis of nanoparticles. We report the synthesis of antibacterial Silver nanoparticles using leaf extract of the medicinal plant, *Tinospora cordifolia*. The leaf extract was prepared by boiling chopped leaves of *Tinospora cordifolia* in deionized water for 10 min and filtering the mixture with Whatman filter paper No.1. The filtrate was used as a reducing agent and stabilising agent for $AgNO_3$. On adding 1 mM solution of Silver nitrate to the leaf extract and stirring at 75 °C for 25 min, a change in colour from yellow-brown to brown-black specified the production of Silver nanoparticles. The formation of Silver nanoparticles was monitored by UV-visible spectroscopy and further characterization of the synthesized Silver nanoparticles was done by XRD studies. The antibacterial studies were performed on Gram negative and Gram positive pathogens, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Staphylococcus aureus*, by agar well diffusion method, on Mueller Hinton agar medium. The Silver nanoparticles synthesized from *Tinospora cordifolia* leaf extract were found to have antimicrobial activity against these Gram negative and Gram positive pathogenic bacteria.

Keywords: Silver nanoparticles (SNP), *Tinospora cordifolia*, characterization, antimicrobial activity.

Síntese fitoassistida de nanopartículas de prata utilizando extrato de folha de Tinospora cordifolia e sua atividade antibacteriana: Uma abordagem ecologicamente correta

Resumo

Para atender à crescente demanda por nanopartículas comerciais, novos métodos de síntese ecologicamente corretos estão sendo descobertos. A síntese de nanopartículas mediada por plantas oferece síntese extracelular fácil e em etapa única de nanopartículas. Relatamos a síntese de nanopartículas de prata antibacterianas utilizando extrato de folhas da planta medicinal *Tinospora cordifolia*. O extrato de folhas foi preparado fervendo folhas picadas de *Tinospora cordifolia* em água deionizada por 10 min e filtrando a mistura com papel de filtro Whatman No.1. O filtrado foi utilizado como agente redutor e agente estabilizante para $AgNO_3$. Ao adicionar solução 1 mM de nitrato de prata ao extrato da folha e agitar a 75 °C por 25 min, uma mudança na cor de amarelo-marrom para marrom-preto especificou a produção de nanopartículas de prata. A formação de nanopartículas de prata foi monitorada por espectroscopia UV-visível e a posterior caracterização das nanopartículas de prata sintetizadas foi feita por estudos de XRD. Os estudos antibacterianos foram realizados em patógenos Gram negativos e Gram positivos, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* e *Staphylococcus aureus*, pelo método de difusão em poço de ágar, em meio ágar Mueller Hinton. Descobriu-se que as nanopartículas de prata sintetizadas a partir do extrato da folha de *Tinospora cordifolia* possuem atividade antimicrobiana contra essas bactérias patogênicas Gram negativas e Gram positivas.

Palavras-chave: nanopartículas de Prata (SNP), *Tinospora cordifolia*, caracterização, atividade antimicrobiana.

1. Introduction

Nanoparticles (NPs) are quite unique in nature because nano size increases surface to volume ratio and furthermore they differ in their physical, chemical and biological properties from bulk material. Nanotechnology is basically a manipulation of matter at the molecular and atomic levels to craft a new structure, device and system with superior electronic, optical, magnetic, conductive and mechanical properties (Khan et al., 2019).

Various approaches are used for synthesis of nanoparticles including chemical, physical, and biological approaches. The physical and chemical methods are the traditional approaches to synthesize NPs. As these methods involve application of sophisticated machinery, high-end equipment and hazardous materials, the process of synthesis is expensive, and causes detrimental effect on the environment (Jain et al., 2021). Chemical synthesis of nanoparticles involve the use of chemicals which are toxic and lead to formation of non-eco-friendly byproducts. Furthermore, in the chemical method of synthesis, capping agents are also required for stabilization of size of the nanoparticles. The need for environment savvy protocols for nanoparticles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals as byproducts. Thus, there is an increasing demand for green nanotechnology (Narayanaswamy et al., 2015). In addition, the biological synthesis of metallic nanoparticles is inexpensive, single step and involves eco-friendly methods.

Nanobiotechnology is the most active area of research in modern material science owing to its multidisciplinary role. Many biological approaches for synthesis of nanoparticles have been reported using microorganisms including bacteria (Dixit; Shukla, 2020; Das et al., 2014; Elbeshehy et al., 2015; Babu et al., 2009), fungi (Mekky et al., 2021; Jain et al., 2011), and plants (Zhang et al., 2022; Phanse et al., 2022; Prajwala et al., 2021).

Plant-mediated synthesis of nanoparticles is an emerging out process in the field of nanobiotechnology (Rajeshkumar et al., 2018). Among the various biological nanoparticles, those produced by medicinal plants have been found to be pharmacologically active. As plants are free from toxic chemicals and possess natural capping agents, they provide a better platform for nanoparticle synthesis. Various parts of plants viz. root, stem leaf, flower, fruit, and seed are utilized for synthesis of nanoparticles.

The biological compounds in the plant parts act as reducing as well as capping/stabilizing agents during the synthesis of nanoparticles. Thus no additional reducing agent needs to be added in the green synthesis of nanoparticles. Especially, the existence of phytochemicals like flavonoids, saponins, tannins, terpenoids, and phenols in plant extracts help in the reduction of metal ions to form metal nanoparticles (Rasheed et al., 2017).

Plants are the major natural resources used for synthesis of the environmentally benign Silver nanoparticles (AgNPs) synthesis. Due to their antimicrobial actions on pathogenic bacteria, Silver nanoparticles are actively involved in medical sciences. Silver nanoparticles show effective antimicrobial activity against pathogenic Gram-positive and Gram-negative bacteria, including multi-drug resistant pathogens (Baptista et al., 2018; Barros et al., 2018).

Tinospora cordifolia (Family: Menispermaceae), a shrub that is native to India, is locally known as *Giloy* and *Guduchi* in Sanskrit. Its root, stems and leaves are used in Ayurvedic medicine and has shown immense application in the treatment of various diseases. It is shown to possess pharmacological actions like anticancer, antiulcer, anti-ischemic, cardiotonic, anti-inflammatory, antispasmodic, antiarthritic, analgesic and diuretic properties (Mukherjee et al., 2022). The pharmacological actions are due to the presence of major phytoconstituents viz., tinosporaside, tinosporone, tinosporic acid and cordifolisides A to E. It is also a source of natural antioxidants (Prasad; Srivastava, 2021). Phytochemicals present include alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds, polysaccharides (Jayaseelan et al., 2011). *Tinospora cordifolia* has been found to exhibit antimicrobial property, against pathogenic microorganisms (Agrawal; Priyadarshini, 2019). Moreover, the AgNPs synthesized from extracts of this plant, enhance the therapeutic efficacy and strengthen the medicinal values of *T. cordifolia* (Singh et al., 2014).

Considering the significance of *T. cordifolia* in various medical applications, the present study has reported an eco-friendly, rapid and easy method for synthesis of Silver nanoparticles (AgNPs) using the aqueous leaf extract of *Tinospora cordifolia* as a reducing and capping agent and evaluation of its medicinal efficacy as an antibacterial agent.

2. Materials and Methods

2.1 Specimen collection and identification

Leaves of *Tinospora cordifolia* were collected during the summer and rainy season (May-June 2023) from *T. cordifolia* plant located in botanical garden of PMB Gujarati Science College, Indore, India. The plant was identified by Prof. Sudip Ray, Head, Department of Botany, PMB Gujarati Science College, Indore. A voucher specimen (Herbarium/Bot.PMB./Micro.-10-2023) of the plant was deposited at herbarium, Department of Botany, PMB Gujarati Science College, Indore. Samples were taken to the laboratory in sealed plastic bag and stored at 4 °C.

2.2 Preparation of leaf extract

Fresh leaves of *T. cordifolia* were washed thoroughly with deionized water. 20 g of leaves were dried, cut into fine pieces added to 100 mL deionized water in 250 mL beaker and boiled for 10 min. The mixture was then filtered with Whatman filter paper No.1 to remove insoluble fractions and macromolecules. The filtrate was used as a reducing agent and stabilising agent for Silver nitrate. The extract was stored at 4 °C for further experiments (Singh et al., 2014).

2.3 Synthesis of Silves nanoparticles

1 mM solution of 80 mL Silver nitrate (Rankem, India, AR Grade, Purity: > 99%) was added to 20 mL of the leaf extract and stirred on a stirring water bath (Remi Water bath shaker, RSB-12) at 75 °C for 25 min. (Prajwala et al., 2021; Singh et al., 2014). A change in colour from yellow-brown to brown-black specified the production of Silver nanoparticles. The solution was sonicated using ultrasonic processor (Rivotek, India) and then the synthesized nanoparticles were collected through centrifugation performed in a cooling centrifuge (Remi C-24, India) (Ashraf et al., 2019). The aqueous plant extract acts as reducing and stabilizing agent for 1mM of $AgNO_3$. The prepared AgNPs were further characterized.

2.4 UV-Visible spectrum analysis

The bio-reduction of Ag ions into Ag^0 was monitored by measuring UV-*Vis* spectrum of the reaction mixture by diluting an aliquot of 0.1 mL of sample into 2 mL of deionized water, within the range of 300-600 nm using UV-*Vis* spectrophotometer (Model UV 1800 Shimadzu, Japan).

2.5 X- Ray Diffraction (XRD)

The nanoparticles were characterized using X-ray diffraction (PANanalytical X'PRO make, USA) with Cu-Kα (wavelength, λ = 1.54 Å) and at an applied voltage of 45 kV and a current of 30 mA. The scanning was performed in the range of 10° - 90° at a step size of 0.02° and exposure of 30 s. To ensure a high signal-to-noise ratio, a zero-background stub (supplied by the manufacturer) was employed. The powder was spread uniformly on this stub material to obtain the information from the large number of powder particles.

2.6 Antibacterial study of Silver nanoparticles

The antibacterial studies were performed on Gram-negative and Gram-positive pathogens, *Salmonella typhi, Pseudomonas aeruginosa, Enterobacter aerogenes and Staphylococcus aureus*, by agar well diffusion method, on Mueller Hinton agar medium, (HiMedia, India). Silver nanoparticles along with $AgNO_3$ and distilled water as control were added into the 6 mm diameter well and incubated for 24 h at 37 °C. The diameter of the inhibition zone formed was measured in mm using digital antibiotic zone reader (EI, India).

3. Results and Discussion

3.1 Synthesis of Silver nanoparticles

When the aqueous leaf extract of *T. cordifolia* was mixed with 1mM $AgNO_3$ solution, the colour changed from yellow-brown to dark brown-black because of the apparent excitation of the plasmon resonance (Phanjom et al., 2012). This colour change is due to the property of quantum confinement which is a size dependent property of nanoparticles that affects their optical property. This is the primary method to confirm that Silver nanoparticles are synthesized (Figure 1).

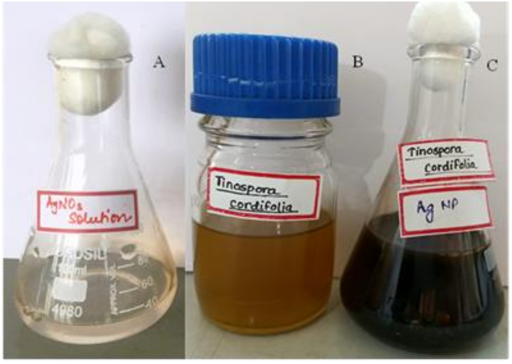

Figure 1. Colour change in reaction mixture (A) 1mM $AgNO_3$. (B) *Tinospora cordifolia* leaf extract. (C) Silver nitrate + *Tinospora cordifolia* leaf extract after 25 min of incubation (reduction of Ag^+ into AgNPs). Source: Authors, 2023.

The colour change is due to the reduction of Silver ions. Bio-molecules present in the leaf extract donate electrons to Ag^+ ions & they are reduced to form Silver nanoparticles. The appearance of dark brown-black colour in the reaction vessels suggest the formation of Silver nanoparticles. Silver nitrate is used as reducing agent as Silver has distinctive properties such as good conductivity, catalytic and chemical stability.

The time duration of change in colour varies from plant to plant. *Tinospora cordifolia* leaf extract acts as a reducing agent and reduces Silver ions (Ag^+) to form a neutral Ag^0 atom. As the concentration of these neutral ions increases, the solution becomes supersaturated and the neutral Ag^0 atoms aggregate to form Silver-nanoparticles. The present study has reported the synthesis of Silver nanoparticles using aqueous leaf extract of *T. cordifolia* from 1 mM $AgNO_3$, without the use of toxic chemicals.

3.*2 UV-Visible spectra analysis*

UV–visible spectroscopy is an important technique to determine the formation of metal nanoparticles in an aqueous solution. The synthesis of Silver-nanoparticles from leaf extract of *T. cordifolia* was confirmed by measuring the UV-*Vis* spectrum of the reaction media. The UV-*Vis* spectrum of colloidal solutions of Silver-nanoparticles have absorbance peaks at 400 to 450 nm (Begum et al., 2019). Visually, the formation of Silver nanoparticles was confirmed by observing the colour change from yellow-brown to dark brown-black colour.

Reduction of Silver ions was monitored by using UV-*Vis* spectroscopy from 300 to 600 nm scan range. The peak obtained at 425 nm (Figure 2) is a typical absorption peak for metallic nanoparticles which further confirms the reduced nanoparticles are Silver. The occurrence of the peak at 425 nm is due to the phenomenon of surface plasmon resonance, which occurs due to the excitation of the surface plasmons present on the outer surface of the Silver nanoparticles which gets excited due to the applied electromagnetic field (Pradhan, 2013).

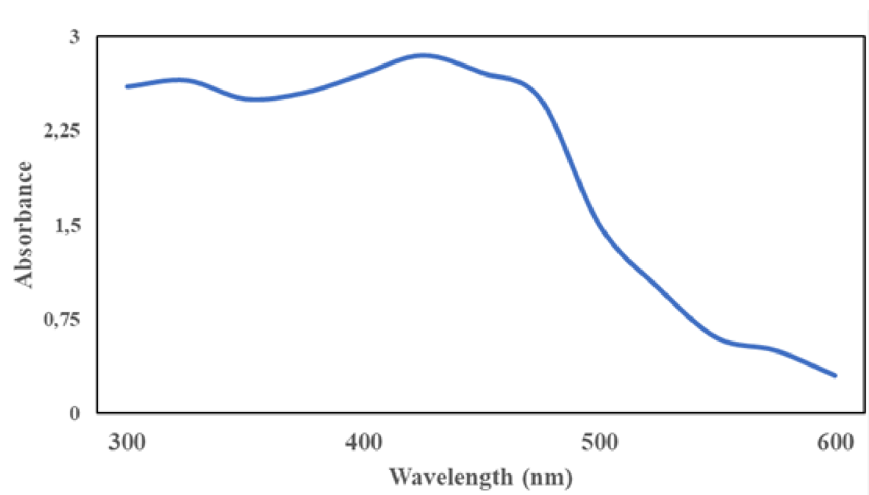

Figure 2. UV-*Vis* absorption spectrum of biosynthesized AgNPs of *Tinospora cordifolia* at a range of 300-600 nm. Source: Authors, 2023.

Silver nanoparticles are efficient at absorbing and scattering light. The strong interaction of the Silver nanoparticles with light occurs because the conduction electrons on the metal surface undergo a collective oscillation when excited by light at specific wavelengths. This is known as surface plasmon resonance (SPR). This oscillation results in strong scattering and absorption properties. Spherical Ag nanoparticles with diameter smaller than about 100 nm show a plasmonic resonance in the UV-*Vis* spectra, centred between 400 and 450 nm depending on the size.

The absorbance peak was formed due to the reduction of Silver ions and characteristic of the SPR, thus indicating the formation of Silver nanoparticles (Daisy; Saipriya, 2012). During the synthesis of Silver nanoparticles, the reaction mixture changes the colour due to the reduction of Silver ions. The reducing agents present in reaction mixture, donate electrons to Ag^+ ions & they are reduced to form Silver nanoparticles.

3.3 XRD analysis

X-ray diffraction (XRD) patterns of AgNPs indicate that the structure of AgNPs is the face centered cubic (FCC) structure of metallic Silver (Figure 3 and Table 1 and, Table 2). The diffraction peaks were observed at 2θ values of 29, 38, 65 and 78. These peaks are due to the organic compounds which are present the extract and responsible for Silver ions reduction and stabilization of resultant nanoparticles. Williamson-Hall grain diameter was calculated to be 6.32 nm.

Similar X-ray diffraction studies for characterization of Silver nanoparticles synthesized from *T. cordifolia* leaf extract were performed by Sevlan et. al. (2017). They reported that the structure of AgNPs was the face centered cubic (fcc) structure of metallic Silver and observed diffraction peaks at 2θ values of 38.1, 44.3, 64.4, and 74.3.

Jha & Prasad (2010) performed XRD studies to ascertain the crystal structure of Ag NPs synthesized from *Cycas* leaves and reported the Rietveld refinements on the XRD data of AgNPs. Their XRD analysis also indicated that AgNPs had a face-centered cubic (fcc) unit cell having the sets of lattice planes (111), (200), (220), (311), and (222).

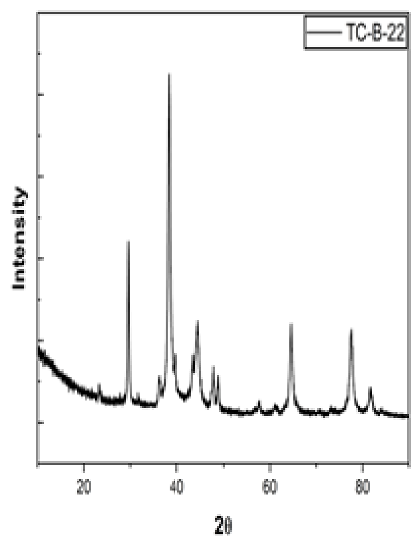

Figure 3. XRD pattern of synthesized AgNPs of *Tinospora cordifolia*. Source: Authors, 2023.

Table 1. XRD analysis of Silver nanoparticles synthesized from *Tinospora cordifolia* leaf extract.

Silver nano-particle have face centered cubic (FCC) structure (Space group: Fm-3m)			
a (nm)	**V (nm^3)**	**D_{W-H} (nma)**	**Strain**
0.4091	0.0685	6,32	+0.0037 (Compressive)

Note: a = Lattice parameter. V = Unit cell volume. DW-H = Williamson-Hall grain diameter. Source: Authors, 2023.

Table 2. XRD analysis: Rietveld refined R, shape parameters.

R_{wp}	R_{exp}	R_p	GOF	u	v	w
37.16	13.20	24.61	2.81	0.037	0.028	0.045

Note: R_{WP} = weighted profile R-factor. R_{exp} = expected R factor. R_p = residual of least-squares refinement. GOF = goodness of fit. Source: Authors, 2023.

3.4 Antibacterial study of Silver nanoparticles

The phyto-synthesised Silver nanoparticles showed antibacterial activity against Gram-negative and Gram-positive pathogens, *S. typhi*, *P. aeruginosa*, *E. aerogenes* and *S. aureus* (Figure 4).

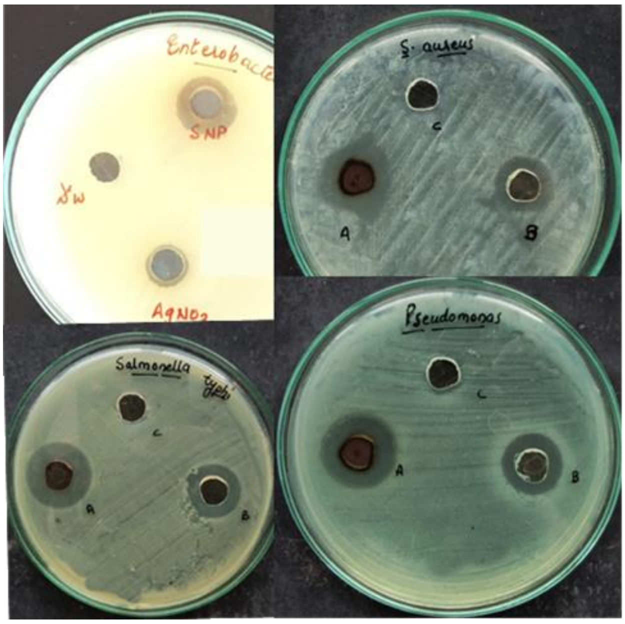

Figure 4. Antibacterial activity of AgNPs synthesized from *Tinospora cordifolia* against pathogenic Gram-negative and Gram-positive bacteria. (A) Zone of inhibition produced by AgNPs. (B) Zone of inhibition produced by $AgNO_3$. (C) Control, sterile distilled water. Source: Authors, 2023.

For the bacterial pathogens, *S. aureus* and *P. aeruginosa*, the inhibition zones obtained due to the antibacterial activity of the synthesized Silver nanoparticles were of 21 mm diameter, each, while the inhibition zones obtained due to the antibacterial activity of 1mM Silver nitrate solution were 17 mm each. In case of *S. typhi*, zone of inhibition given by the synthesized Silver nanoparticles measured 20 mm and that by 1mM Silver nitrate solution was 15 mm. For *E. aerogenes*, zone of inhibition given by the synthesized Silver nanoparticles measured 17 mm and that by 1mM Silver nitrate solution was 12 mm.

Thus, in the present study, the synthesized Silver nanoparticles showed inhibition against both Gram positive and Gram negative bacterial pathogens with maximum inhibition against *S. aureus* and *P. aeruginosa* followed by *S. typhi* and *E. aerogenes*. Various studies have been done by many researchers which confirm that *T. cordifolia* was found to be good antibacterial agent against pathogenic and non-pathogenic organisms (Duraipandiyan et al., 2012, Rajathi et al., 2012).

Our results are in concurrence with studies carried out by Rachel et al. (2020), who reported antimicrobial activities of Silver nanoparticles synthesized from extracts of *T. cordifolia* against pathogenic bacteria like *Staphylococcus* sp., *Enterococcus* sp., *Pseudomonas* sp., *Streptococcus* sp. and *Klebsiella* sp. (Rachel et al., 2020). Similarly, as reported by Anuj (2013), Silver nanoparticles from stem powder extracts of *T. cordifolia* have been known to have bactericidal and inhibitory effects and were effective against *S. aureus*, *P. aeruginosa* and *Escherichia coli* (Anuj, 2013). In another study by Selvam et al. (2017), Silver nanoparticles synthesized using extracts of *T. cordifolia* have been known to possess antibacterial activity and were effective against *S. aureus* and *Klebsiella* sp. (Selvam et. al., 2017).

4. Conclusions

The present study included the bio-reduction of Silver ions through the leaf extract of the medicinal plant, *Tinospora cordifolia*, and testing for their antimicrobial activity. Biosynthesis of AgNPs using green resources like *T. cordifolia* is a better alternative to chemical synthesis, since it is simple, ecofriendly and economic. The present investigation revealed that the *T. cordifolia* leaf extract would be a good source for green synthesis of Silver nanoparticles.

The phyto-synthesised Silver nanoparticles showed antibacterial activity against Gram-negative and Gram-positive pathogens, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Enterobacter* and *Staphylococcus aureus*. The bactericidal study of these nanoparticles may play a vital role in the treatment and invention of new drugs against infections caused by *S. typhi*, *P. aeruginosa*, *Enterobacter* and *S. aureus*.

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6. Authors' Contributions

Conceptualization of the work and general methodology was contributed by *Nandini Phanse* and *Krishnaiah Venkataraman*. *Nandini Phanse* performed the biological synthesis and antimicrobial activity of Silver nanoparticles. *Krishnaiah Venkataraman*, *Pravin Kekre*, *Sanjay Shah* and *Shilpa Parikh* contributed in the physicochemical analysis of synthesized nanoparticles. Validation of XRD studies was done by *Krishnaiah Venkataraman*. *Nandini Phanse* and *Shilpa Parikh* contributed in drafting of manuscript and computation of data.

7. Conflicts of Interest

No conflicts of interest.

8. Ethics Approval

Not applicable.

9. References

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