



REVIEW ARTICLE

Biogenesis of nanoparticles using microorganisms: A Review

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Abstract

Bionanoparticles are synthesized using novel strategies through environmentally benign approaches. Emphasis is on synthesizing nanoparticles using green chemistry principles to reduce the burden of pollution on the environment. The biological approach for the synthesis of metallic nanoparticles is also described as green synthesis (bioprocess) of nanoparticles, is now being looked at as an alternative to physio-chemical approaches and generally uses biological components like plants and microbes (bacteria, fungi, algae and yeast) and cause minimal harm to the nature. The naturally occurring potential biodegradable agents like enzymes (secreted by microbes) act as reducing agents and play a very distinct role in the synthesis of nanoparticles. Most bioprocesses occur under normal air pressure and temperature, resulting in vast energy savings and reducing the use of expensive chemicals making the green approach less costly. This process of synthesis of nanoparticles using biological systems is referred to as nanobiotechnology. Nanobiotechnology has emerged as an integration between biotechnology and nanotechnology for developing biosynthetic and environmentally friendly technology for nanoparticle synthesis. This review is mainly focused on the microbial synthesis of nanoparticles utilizing the extract of bacteria and algae. In the present review, the bio-reduction capacity of various bacteria and algae is highlighted in detail, which has yet to be discussed earlier. This is a comprehensive work underlining the synthesis of nanoparticles, their bio-reduction ability, and application of nanoparticles.

Keywords

bionanoparticles, bacteria, algae, biosynthesis, microbes

Introduction

The ever-increasing pollution in today's world has necessitated using environment-friendly technologies for nanoparticle production and to reduce the various environmental hazards in the process (1). Nanotechnology involves the synthesis of particles at the molecular level and characterization along with their application in various fields (2). The use of green technology to synthesize nanoparticles is needed in today's world. Here, bionanoparticles are synthesized using biological/green products, proving the process to be environment-friendly and cost-effective, reducing the usage of toxic and costly chemicals. Nanoparticles are particles with at least one measurement less than 100 nm (3). The nanoparticles synthesized biologically are defined as bionanoparticles, and they are known to occur naturally, either intracellularly or extracellularly, depending on the place of their formation in plants and microbes (4).

Nanoparticles demonstrate unique chemical, physical, magnetic, optical, and biological properties (improved antimicrobial and anti-

inflammatory activity) as they have increased surface area to volume ratio (3,5). These properties exhibit tremendous utilised in chemical industries, metallurgical sector, energetic, and agriculture (3). In addition, metallic nanoparticles also exhibit surface plasmon resonance (SPR) in the visible region of UV-visible spectrophotometer (6). Surface plasmon resonance is defined as the collective oscillation of electrons in the metal surface when hit by certain incident light with a certain angle of incidence, giving rise to the SPR band appearing in the visible region (7).

Nanoparticles are majorly grouped into two classes: organic and inorganic. The organic ones include nanoparticles of carbon such as nanotubes, mainly cylindrical shapes, and spherical buckyballs, known as fullerenes (8). Magnetic, noble metal (gold, silver, copper, palladium, and platinum) and semiconductor nanoparticles like Zinc sulphite, cadmium sulphite, and Zinc oxide (ZnS, CdS, and ZnO) are examples of inorganic nanoparticles (9). Among them largest market share is occupied by nanotube of carbon, fullerenes, and metallic nanoparticles with a wholesale price range of 4 to 18 €g⁻¹ (3). Magnetic nanoparticles can be manipulated by means of a magnetic field and contains magnetic elements such as nickel, cobalt, iron, and their chemical compounds. The noteworthy properties of metallic nanoparticles give rise to promising applications in various fields such as medical field, cosmetics, optical industries, chemical industries, wastewater treatment, etc. (2). Metallic nanoparticles are known to show size and shape-dependent properties such as catalyst, antibacterial activity, sensors, etc. (10). For instance, gold and silver nanoparticles have promising applications in biomedical field for example gold nanoparticles are used for protein detection, immunoassay, protein assay, detecting cancer cells and capillary electrophoresis (11). The silver nanoparticles have high antimicrobial activity and are used in health, medicine, cosmetics, packaging, animal husbandry, etc. It is evident that smaller the size of silver nanoparticles, the higher the antibacterial properties (10). Similarly, the catalytic activity of other metallic nanoparticles increases as the size decreases. They have a high antimicrobial effect against various microbes such as *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *S. epidermidis*, and *Klebsiella pneumoniae* (11,12). Despite huge applications in the medical field, silver nanoparticles are also extensively used in integrated circuits, sensors, bio-labeling, filters, antimicrobial deodorant fibers, cell electrodes, etc. (13). Nanoparticles also find their application in environmental remediation, where they are used as a catalyst in degradation of toxic textile dyes (14). Nanoparticles of noble metals have huge biomedical applications. Semiconductor nanoparticles are utilized in biological labelling, barcodes, and fluorescent materials for display screens (8). Nanoparticles of gold, silver, selenium, platinum, palladium, silica, titania, zirconia, and uraninite are mainly synthesized biologically.

Nanoparticles are synthesized by various physical and chemical procedures. Electrochemical, chemical reduction, photochemical, and physical vapor

condensation are mostly used processes (3). Various disadvantages of physio-chemical processes are excess cost, high energy consumption, complicated reactions, and toxic chemicals. Further, these processes achieve high purity of 99.995% with perfect shapes but high production costs, almost 90% of the selling price, hampers their development commercially (3). Thus, finding a suitable alternative to synthesize nanoparticles using biological methods is necessary. This involves synthesizing biological material/biomass at ambient pressure and temperature conditions without harmful chemicals. Synthesis of nanoparticles using microbes is new, but the interrelationship between microbes and metal ions has been known for several decades for their ability to accumulate metals in bioleaching and bioaccumulation (2). Various plant products and microbes are used as sources of biological material for biosynthesizing nanoparticles.

Microbes are tiny creatures with huge potential to synthesize nanoparticles in an eco-friendly manner and with a high success rate. Many researchers have mentioned the use of bacteria, fungi, algae, and yeast for the biosynthesis of nanoparticles. Several extracellular enzymes (nitrate reductase), naphthoquinones, and anthraquinones present in microbes behave as a reducing agent for reduction of metal salt to nanoparticles. The use of microbes in bionanoparticles synthesis is a rapidly growing field due to the ease of formation of nanoparticles and cost-effectiveness. Therefore, microbial synthesis of nanoparticles can be an effective and economical alternative to synthesize nanoparticles at a large scale.

Following above, our review is hypothesized to synthesize nanoparticles using green technologies considering the current scenario of increasing environmental pollution. The applications of bionanoparticles synthesized in a sustainably and economically way using bacterial and algal species have been highlighted.

Microbial synthesis of nanoparticles:

Biological synthesis of nanoparticles is receiving tremendous attention now a day. During synthesis, biological reducing agents reduce metal salt to nanoparticles. The non-hazardous and eco-friendly plants and the microbial (bacteria, actinomycetes, viruses, fungi, algae, yeast) extracts are the main sources for the synthesis of bionanoparticles (15). Besides using green biomass, the other benefit of biosynthesis is that reaction is carried out in aqueous media, which is environment-friendly at ambient temperature and pressure. Microbes are considered to be nano-factories with noteworthy capabilities for synthesizing nanoparticles under controlled conditions (4). An intrinsic mechanism in microbial systems synthesizes nanoparticles from metallic salts (16). Microbial cells play an important role in reducing heavy metals to metallic nanoparticles. Microbial cells possess an advantage over others in that they can produce environmentally benign and sustainable nanoparticles at large scale with rapid growth and simple mechanism (17, 18). The bacterial species can survive at higher

concentrations of metallic ions and metal salts (17). During the microbial synthesis of nanoparticles, first, metal ions are confined at the cell's surface, and finally, the reducing agent (enzymes) reduces the metal ions to nanoparticles (19). The intracellular process is more complicated as it requires one more step of expulsion of formed nanoparticles from the cell.

Synthesis of nanoparticles generally involves three main components during microbial synthesis: (1) Salt of a metal particle (usually a solvent medium). (2) Reducing agent: Micro-organisms culture extract It is majorly the microbial enzymes/metabolites (e.g., - nitrate reductase from a fungus (*Fusarium oxysporum*), also several naphthoquinones and anthraquinones (20) with reducing properties that act on the respective compounds and give the desired nanoparticles. (3) Stabilizer (capping agent): Chemical methods involve the use of organic solvents as stabilizing agents (which are very harmful). However, in the biosynthesis method, the bioorganism acts as both, a reducing agent and a capping agent (stabilizing agent), thus making the product safer (21).

Biosynthesis using Bacteria: Bacteria belong to prokaryotes, the largest group of unicellular living organisms found everywhere (soil and water) and in symbiosis with other organisms. Commonly nanoparticles are synthesized extracellularly using bacteria (2). The genus *Bacillus* and *Pseudomonas* are extensively used for the biosynthesis of nanoparticles in living or dead conditions (2). Gold nanoparticles have been synthesized using *Rhodococcus* sp., *Pseudomonas aeruginosa*, *Shewanella oneidensis* (22-24) (Table 1). Silver nanoparticles are well known for their antimicrobial activity against various Gram-positive and Gram-negative bacteria such as *Escherichia coli*, *Bacillus subtilis*, *Acinetobacter* sp., *Staphylococcus aureus*, *S. epidermidis*, etc. Ganesh Babu & Gunasekaran (25) (Table 1)

synthesized spherical silver nanoparticles with a 4-5 nm size range using metallic resistant bacterial strain isolated from the electroplating industry. Gurunathan et al. (26) reported a reduction of silver metal salt to trigonal or hexagonal silver nanoparticles (50 nm) using culture supernatant of *E. coli* (Table 1). Oxide nanoparticles (magnetic and non-magnetic) have also been synthesized using bacteria. *Aquaspirillum magnetotacticum* has been used for the synthesis of octahedral prism shaped Fe_3O_4 nanoparticles with a size range of 40-50 nm (27) (Table 1). Hassan & Mahmood (28) synthesized spherical Fe_2O_3 nanoparticles (27.7 nm) using a culture supernatant of *E. coli* (Table 1). Some examples of nonmagnetic oxide nanoparticles are TiO_2 , CuO , ZnO , Sb_2O_3 , SiO_2 , $BaTiO_3$, and ZrO_2 . *Bacillus amyloliquefaciens* has been used for the synthesis of TiO_2 nanoparticles having a spherical shape with a size range of 22.11-97.28 nm (29). CuO nanoparticles have been synthesized using *Trichoderma asperellum* (30), and *Periconium* sp. has been used for ZnO nanoparticles (31) (Table 1). Sweeney et al. (32) synthesized sulphide nanoparticles (with wurtzite crystal phase, size 2-5 nm) through *E. coli* (Table 1). Nanoparticles of ZnS and PbS are successfully synthesized using immobilized *Rhodobacter sphaeroides* with an average particle size of zinc sulphide (8nm) and lead sulphide (10.5 nm) (33-34) (Table 1).

Biosynthesis using Algae: Algae are thaloid plants ranging from microscopic to macroscopic levels and are utilized to synthesize nanoparticles. Algae are easily accessible to collect, as they are found naturally in the lentic ecosystem, such as ponds, lakes, and oceans (35). Microalgae comprise high protein levels and high lipid content, especially omega-3 fatty acids, which can be a reducing agent for synthesizing of bionanoparticles (36). The macroscopic algae have an advantage over the microscopic algae due to their high metal uptake capacity. *Chlorella vulgaris* and *Sargassum wightii* have been

Table 1. Bacterial mediated biosynthesis of nanoparticles.

Name of the bacteria	Average size (TEM/SEM/XRD)	Morphology/Shape of AgNPs	Absorption peak in UV-visible spectrophotometer	Name of the nanoparticles	Reference
<i>Aquaspirillum magnetotacticum</i>	40-50 nm	Octahedral prism	-	Fe_3O_4	(27)
<i>Bacillus amyloliquefaciens</i>	22-97 nm	spherical	-	TiO_2	(29)
<i>Bacillus cereus</i>	4-5 nm	spherical	420 nm	Ag	(25)
<i>Escherichia coli</i>	50 nm	Triangular, hexagonal	420	Ag	(26)
<i>Escherichia coli</i>	2-5 nm	Spherical, elliptical	-	CdS	(32)
<i>Escherichia coli</i>	67 nm	spherical	473 nm	Fe_2O_3	(28)
<i>Periconium</i> sp.	16-78 nm	Hexagonal	-	ZnO	(31)
<i>Pseudomonas aeruginosa</i>	15-30 nm	polygonal	-	Au	(23)
<i>Pseudomonas putida</i>	12-30 nm	irregular	-	CdS	(58)
<i>Rhodobacter sphaeroides</i>	8 nm	spherical	-	ZnS	(33)
<i>Rhodobacter sphaeroides</i>	10.5 nm	spherical	-	PbS	(34)
<i>Rhodococcus</i> sp.	13 nm	spherical	540 nm	Au	(22)
<i>Shewanella oneidensis</i>	2-50 nm	spherical	-	Au	(24)
<i>Trichoderma asperellum</i>	10-190 nm	spherical	285-295 nm	CuO	(30)

reported for the biological synthesis of gold nanoparticles (37,38) (Table 2). *Chaetoceros calcitrans*, *Tetraselmis gracilis*, *Isochrysis galbana*, and *Chlorella salina* are used to synthesize silver nanoparticles (39) (Table 2). These silver nanoparticles are found to have good antimicrobial activity against selected strains of *Escherichia coli*, *Proteus* sp., *Pseudomonas* sp., and *Klebsiella* sp. (39) (Table 2). Both magnetic and non-magnetic oxide nanoparticles have also been synthesized using algae. Magnetic nanoparticles (Fe_3O_4) have been synthesized through brown and red seaweed from the Mediterranean Sea, Egypt (40,41). Non-magnetic oxide nanoparticles of CuO (5-45nm; spherical) have been obtained through extracts of brown algae such as *Bifurcaria bifurcate* (42) (Table 2). *Spirulina (Arthrospira) platensis* is used for the synthesis of spherical CdS nanoparticles with a size range of 8-12 nm (43) (Table 2).

Mechanism of nanoparticles synthesis

The formation of nanoparticles by microbes is a significant part of geo-cycles and occurs specifically as a stress response ensuing in defence against metal poisoning (44). Thus, the microorganisms able to synthesize nanoparticles are generally metal resistant. It is reported that contact of negative anionic cell constituents with positive cationic metal ion lead to the formation of nanoparticles via reduction, chelation, or hydrolysis. To cope up with metal toxicity, the microorganism can take into enzymatic reduction, precipitation, dissimilatory oxidation, complexation, or transport via efflux systems to remove

the metals from the cell (45). The pathways involving enzymes, exopolysaccharides, and proteins are broad mechanisms for synthesizing nanoparticles (44). Nanoparticle synthesis using microbes is advantageous over other greener routes as it gives control over shape and size. Mainly monodispersed nanoparticles are formed, which can be replicated at the industrial level in bioreactors. The size and shape can be controlled by varying the external parameters such as pH and temperature. Enzymes are the most prominent things responsible for reducing metal to nanoparticles via redox reactions occurring inside (intracellular) and outside (extracellular) microbial cells. The exact mechanism of nanoparticle synthesis by biological extracts is difficult and has yet to be understood thoroughly. Reductases, naphthoquinones, flavonoids and, anthraquinones, and biomolecules such as amino acids, peptides, proteins, enzymes, aromatic and aliphatic compounds are the main reducing agents which reduce metal ions to nanoparticles form in microbes (46). Several polysaccharides, peptides, pigments in algae, and discrete enzymes found in the bacterial cell, like NADH-dependent reductase or nitrate-dependent reductase, play the role of reducing agent. The metal salt of a nanoparticle is reduced by reducing agents or enzymes present in microbes. The metal is present in (M^+) form in metal salt, and enzymes provide electrons, reducing the metal cation to metallic form (M^0) or nanoparticles. The reaction can be summarised as:

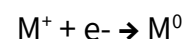


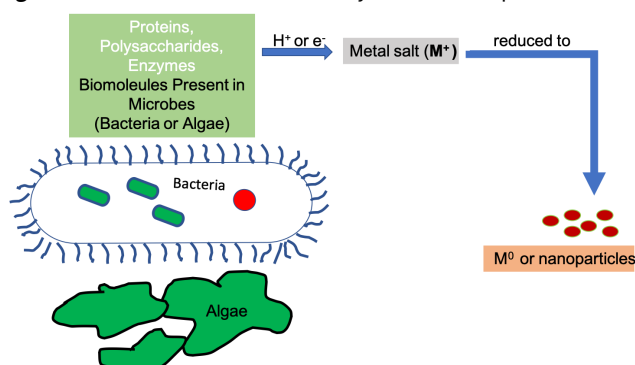
Table 2. Algal mediated biosynthesis of nanoparticles.

Name of the Algae	Average size (TEM/ SEM/	Morphology/ Shape of AgNPs	Absorption peak in UV-visible spectrophotometer	Name of the nanoparticles	Reference
<i>Bifurcaria bifurcate</i> <i>Chaetoceros calcitrans</i> (green algae)	5-45 nm	spherical	260 and 650 nm	CuO	(42)
<i>Chlorella salina</i> (green algae)	53-71 nm	-	420 nm	Ag	(39)
<i>Chlorella vulgaris</i> <i>Chlorococcum</i> sp. MM11 <i>Colpomenia sinuosa</i> (brown seaweed)	2-10 nm 20-50 nm 11.24-33.71 nm	- spherical spherical	530 nm	Au Fe Fe_3O_4	(37) (59) (40)
<i>Isochrysis galbana</i> (green algae)	53-71 nm	-	420 nm	Ag	(39)
<i>Padina pavonica</i> <i>Pterocladia capillacea</i> (red seaweed)	10-19.5 nm 16.85-22.47 nm	- spherical	402 nm 415 nm	Fe_3O_4 Fe_3O_4	(41) (40)
<i>Sargassum acinarium</i> <i>Sargassum bovinum</i> <i>Sargassum wightii</i> Shewanella	21.6-27.4 nm 5-10 nm 8-12 nm 5 nm	- octahedral spherical -	415 nm - 527 nm	Fe_3O_4 Pd Au Pt	(41) (60) (38) (61)
<i>Spirulina (Arthrospira) platensis</i> <i>Tetraselmis gracilis</i> (green algae)	8-12 nm 53-71 nm	spherical -	420 nm 420 nm	CdS Ag	(43) (39)

The mechanism of producing nanoparticles varies with different biological species and location of their formation (either extracellularly and intracellularly) (47).

Either extracellular or intracellular mechanisms are involved in the microbial synthesis of nanoparticles, which mostly requires oxidoreductase enzymes (e.g., NADH-dependent nitrate reductase, NADPH-dependent sulphite reductase flavoprotein subunit α , and cysteine desulfhydrase) and cellular transporters (48). In the extracellular process, synthesis occurs at the cell surface, whereas in intracellular synthesis, nanoparticles form inside the cell in the presence of enzymes. For extracellular synthesis, it is mainly the microbial reductase enzyme, proteins, bacterial cell wall components, and organic molecules in the culture medium responsible for reducing metal ions into nanoparticles. The NADH-dependent enzyme acts as an electron carrier and withdraws electrons from NADH (reducing it to NAD^+) and the available electrons, in turn, reduces metal ions to nanoparticles (46). Extracellular enzymes such as nitrate reductase help in the transfer of electrons from various groups (e.g., hydroxyl groups) to metal salt and thus lead to the formation of nanoparticles (Fig. 1). In the intracellular process, the metal ion needs to be transported inside of the microbial cell, where cell wall plays a very important role. The probable mechanism comprises the electrostatic interaction of negatively charged carboxy group cell walls with positively charged metal ions, resulting in metal ions across the cells and reduction by intracellular proteins to produce nanoparticles (17). Further, intracellular enzymes like cytochrome oxidase also help in reduction of metal ions to nanoparticles with the help of electron transference amongst cytoplasm constituents (e.g., NADH/NADPH), vitamins, and organic acids (49).

Figure 1. Probable mechanism to biosynthesize nanoparticles.



Application of nanoparticles

Bionanoparticles, with their unique properties, find their applicability in medicine, food preservation, catalysis, and environmental remediation or wastewater treatment. Numerous metallic nanoparticles, such as silver, copper, zinc, gold, and titanium, are recognised for their antimicrobial properties. Also, nanoparticles of silver are well-known for possessing high anti-fungal, anti-viral, and anti-inflammatory properties, increasing their applicability in the medical field (16). According to a recent report by Bartoš et al. (50) and Vochozka et al. (51), the pricing of metals plays a key role in the production of nanoparticles

on a commercial scale. It is reported that the price of metals such as copper and aluminum is intended to increase in the coming years (as per the detailed study from 2011 to 2022). The production of aluminium was showing an increasing trend whereas production of copper is known to stagnant due to high price and a limited quantity of copper metal. The growing trend of metal pricing might impede the products made from them, such as nanoparticles. Another report by Rowland et al. (52) described that the price of silver from 2011 to 2022, which is highly prone to the economic cycle of the world economy. The data shows that the price reached 37 USD per ounce in the initial months of 2012 and 35 USD in 2022. The price of zinc was also evaluated, which is expected to increase slightly by 2030 to 410 USD per ton (53).

Magnetic bionanoparticles have distinct features like super paramagnetic and high coercive force with applications in biological separation and biomedical fields due to micro-configurational arrangement. The most common magnetic nanoparticles are Fe_3O_4 (magnetite) and Fe_2O_3 (maghemite). They are biocompatible and actively studied for the treatment of cancer (magnetic hyperthermia), drug delivery, stem cell sorting and manipulation, gene therapy, and magnetic resonance imaging (MRI). Sulphide nanoparticles are semiconductor nanocrystals, gaining widespread attention due to their unique electronic and optical properties in elementary research as quantum-dot fluorescent biomarkers, cell labelling agents, and nanoelectronics. The extracellular nanoparticles have wide applications in optoelectronics, electronics, bio-imaging, and sensor technology as in other intracellular synthesized nanoparticles (15).

Bionanoparticles are used in the food industry as a disinfectant and sterilization. For example, the addition of nanoparticles in coating of food packaging act as an antibacterial agent which helps in disinfecting the food and preventing it from putrefaction (16). Urbanization and industrialization have led to increased pollution in the environment. There are many sources of water pollution, the major being the industries that discharge their untreated effluent into the water bodies. Recently, nanotechnology has been considered a good candidate for treating polluted water by disinfection, purification, and desalination (4).

The high surface area to volume ratio of bionanoparticles also imparts them high catalytic activity. Thus they are applied as nanocatalysts in treatment and degradation of organic pollutants, such as dyes, nitrophenols, etc. Bionanoparticles of silver, Iron, and nano-nickel zinc ferrite catalysts are used for degradation of various toxic and hazardous dyes such as methyl orange, methyl red, methylene blue, malachite green, reactive black, brilliant blue (14,54). Organic compounds such as 4-nitrophenol and its derivatives are used in manufacturing of many herbicides, and insecticides and can considerably damage the ecosystem/nature. This can be reduced by using nanocatalysts such as gold, silver, copper oxide, and palladium in the presence of reducing agents (55).

Nanoparticles are also utilised for the fabrication of biohydrogen, such as nanoparticles of Ag, Au, Cu, Fe, activated carbon, Ni, Pd, SiO₂, Ti, and carbon nano-tubes have been reported. The higher surface area to volume ratio of nanoparticles provide them greater potential to adsorb charged particles, thus stimulating biohydrogen fabrication (56). Recently, nanoparticles of aluminum synthesized from a waste mixture of polyethylene and aluminum (PE-Al) or 'tetra pack' (used for liquid packaging) are utilised to increase the effectiveness of biodiesel (57).

Considering the low production cost compared to conventional methods and remarkable applications of nanoparticles in enormous fields, further research can be attempted to discover the potential of bacterial and algal species for mass production of nanoparticles on a larger scale and the perspective of other microorganisms in the biosynthesis of nanoparticles can also be looked upon.

Conclusion

Microbes are biological entities present in abundance in nature, and synthesizing nanoparticles using them is an eco-friendly process. The bionanoparticles can be synthesized using green chemistry principles and are being looked at as alternative to conventional approaches. The microbial organisms are readily available, and the technique for synthesis is cost-effective. It is a valuable substitute for the extensive production of metal nanoparticles. Further, bionanoparticles have wider applications in various fields like medicine, water remediation, as a catalyst for various biosynthetic reactions, etc., and using them will lower the complete application cost. Also, to fulfill the demand, these bionanoparticles can be synthesized at a large scale with emphasizing low-cost, high-yield production.

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Authors' contributions

AS wrote the manuscript with support from PB and PBG, SG and HC supervised the project.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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