RESEARCH PAPER



Biosynthesis of Silver Nanoparticles from the Mangrove *Rhizophora mucronata*: Its Characterization and Antibacterial Potential

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Abstract

The green synthesis of nano-sized particles with specific functions is of great significance in the present bio-nanotechnology. In this study, the biosynthesis of silver nanoparticles (AgNPs) from the aqueous extract of stem, root and leaf of Rhizophora mucronata, one of the two native mangrove plants in southern Iran, and its activity against some bacterial pathogens are reported for the first time. A green procedure for the synthesis of AgNPs using the mangrove extract as reducing agent was used. Synthesized AgNPs were investigated using UV-visible spectrophotometry, transmission electron microscopy (TEM), field emission scanning electron microscopy, X-ray diffraction (XRD), energy-dispersive spectroscopy (EDS) and Fourier transform infrared (FTIR) spectroscopy. Antibacterial effect of the biosynthesized AgNPs was investigated by agar well diffusion assay against pathogenic bacteria. The leaf extract yielded the highest production of biosynthesized AgNPs among the plant parts. UV-visible spectrophotometry at a wavelength of 420 nm confirmed the synthesis of AgNPs. The results from the XRD analysis approved the formation of crystalline AgNPs with crystallinity percentage of 88.54. As shown by TEM evaluation, AgNPs had the same spherical morphology. The size of the biosynthesized AgNPs was between 1 and 80 nm with average size of 32.44 nm. According to the size distribution histogram, nanoparticles in the size range of 20–25 nm were more than other size groups. SEM images identified silver nanoparticles ranging in size from 10 to 19 nm. Results of EDS showed the existence of carbon, silver, oxygen and chlorine in the nanoparticles synthesized from the leaf extract of R. mucronata. Silver was the major constituent element, 73.5%. FTIR analysis distinguished different functional groups such as aromatic loops, alcohol, phenol group, alkanes and alkyl halides in the biosynthesis process. The biosynthesized AgNPs had potential antibacterial activity against the bacterial pathogens. Out of the selected bacterial strains, the Gram-positive bacteria of Bacillus cereus with the maximum inhibition zone and the Gram-negative bacteria of *Pseudomonas aeruginosa* with the minimum inhibition zone were the most sensitive and resistant bacteria, respectively. The mangrove Rhizophora mucronata extract was found to be a suitable reducing agent for biosynthesis of AgNPs with approved antibacterial activity.

Keywords Rhizophora mucronata · Green synthesis · Biological activity · Silver nanoparticles · XRD · Bacterial pathogens

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1 Introduction

Nanotechnology is a rapidly expanding area of new science that has widespread applications in many natural sciences and industries. One of the goals of this technology is to produce nanoparticles which are a kind of nanomaterials with diverse and controlled properties, size and shape, where it can serve the human beings (Sarkheil et al. 2017; Abdi et al. 2018). Owing to their very small size and the low surface-to-volume ratio, nanoparticles have unique optical, mechanical, thermal, electrical, magnetic and chemical properties (Ghasemi et al. 2016a, b). Metallic



nanoparticles can be potentially used for their remarkable inhibitory and antimicrobial properties. These properties are of particular interest to scientists because the microbial resistance against metal ions and antibiotics has increased and the resistant strains have been developed (Rai et al. 2009). Among different nanoparticles, the silver nanoparticles (AgNPs) have many advantages in nanotechnology, bio-nanotechnology, medicine, industry, agriculture and food, and potentially can be used in human life as an alternative to silver ions (Ahmed et al. 2016; Sarkheil et al. 2016). Studies have shown that AgNPs display more antibacterial and antiviral effects than other nanoparticles and can directly interact with bacterial cells and thus oxidize and ultimately lead to death of bacterial cells (Rai et al. 2009; Ibrahim 2015; Sarkheil et al. 2017).

There are different chemical and physical procedures to synthesize AgNPs in different sizes. Although the chemical and physical methods for nanoparticles production may be successful, they are expensive and environmentally unsafe. The nanoparticles produced by chemical methods use toxic chemicals as reducing and stabilizing agents which are harmful to living organisms, remain non-degraded in nature and ultimately lead to chemical contamination of the environment. Other drawbacks of these methods include high consumption of energy and low yield, solvent contamination and lack of uniform distribution related to physical methods (Mallick et al. 2004; Ibrahim 2015; Zhang et al. 2016). Therefore, researchers are trying to replace it with environment-friendly, cost-effective and chemical-free methods called green synthesis.

The green synthesis or biological production of nanosized particles with specific functions is attracting an increasing amount of interest in bio-nanotechnology. Recently, biosynthesis of nanoparticles by means of plants and microorganisms as a biocompatible and economic method has been of interest to many researchers. Biological methods for synthesizing metal nanoparticles using herbal extracts seem to be a good alternative to physical and chemical procedures, as they play a very crucial role in modifying toxic chemicals by reducing the metal ions (Makarov et al. 2014; Yew et al. 2016). Biosynthesis of AgNPs using microorganisms, fungi, plants and algae instead of chemical and physical methods is of considerable importance (Li et al. 2011; Gopinath and Velusamy 2013; Rajeshkumar et al. 2013). The biosynthesis of AgNPs using plants extract is safe and clean with a simple experimental procedure. In fact, the basis for the synthesis of AgNPs is the reduction of silver ions. In doing so, plants can be widely used without causing environmental damage as they are environmentally compatible (Ahmed et al. 2016; Abdi et al. 2018). In the synthesis process, the extracts of these organisms perform as both reducing agents and coating agents of silver ions for generating AgNPs, so that the reduction of



silver ions through biomolecules including amino acids, proteins, enzymes, tannins, alkaloids, saponins, phenolics, carbohydrates, flavonoids and vitamins found in herbal extracts takes place in an environment-friendly manner (Prakash et al. 2013; Keat et al. 2015).

Mangrove plants are halophyte plants in tropical and subtropical areas in some parts of the world. Different chemical compounds have been extracted from different parts of these plants, including terpenoids, steroids, tannins, naphthalenes, flavonoids, glucosides, glycosides and alkaloids (Patra and Thatoi 2011; Bakshi et al. 2015; Balakrishnan et al. 2016). These compounds have various biological properties such as antiviral, antibacterial, antifungal and anticancer activity (Patra and Thatoi 2011; Syed Ali et al. 2015; Balakrishnan et al. 2016). The Rhizophora mucronata species of mangrove plants belongs to Rhizophoraceae family and has a large distribution in the Indo-Pacific region. The distribution of this native mangrove species in Iran is limited to the saline wetlands between Sirik and Jask ports in the intertidal regions along sheltered coastlines of Hormozgan Province, southern Iran, and does not exist in other parts of the Persian Gulf and Oman Sea region.

Considering the widespread use of nanoparticles and their significance in various fields of science and also the advantages of biological synthesis methods, particular attention has been recently paid to the production of nanoparticles using plants and herbal extracts. Mangrove plants are a candidate for this purpose. Some studies have reported the synthesis of AgNPs by using mangroves extracts. In one of the most recent publications, synthesis of AgNPs using *Avicennia marina* mangrove extract was illustrated and concluded that this biosynthesis is environment-friendly and has the potential to be set up for large-scale syntheses (Abdi et al. 2018).

Regarding the information provided on the biological synthesis of nanoparticles and also the growth of *Rhi-zophora mucronata* species in parts of Hormozgan coasts, southern Iran, as well as the reports provided on the medicinal properties of mangrove plants in the previous literature (Gnanadesigan et al. 2012; Balakrishnan et al. 2016), this study was designed to investigate the biological synthesis of AgNPs employing the native mangrove *Rhi-zophora mucronata* from southern coasts of Iran and to assess its antibacterial activity against some pathogenic bacteria for the first time.

2 Materials and Methods

2.1 Sample Collection and Preparation

To synthesize AgNPs, various parts such as stems, roots and leaves of the mangrove plant *Rhizophora mucronata* were gathered from the Azini wetland located between Sirik and Jask ports in the saline intertidal zone, Hormozgan Province, southern Iran. Collected samples were first washed with seawater, then with tap water and at last with distilled water to eliminate the contaminants. Then, they were shade-dried at room temperature for 2 weeks. A mixer grinder was used to powder the dried specimens.

2.2 Preparation of R. mucronata Extract

A quantity of 100 g of leaf, stem and root powder were separately mixed with 700 mL of deionized water and kept for 10 min at boiling temperature on the heater. After cooling, it was passed through Whatman filter paper (No. 1) and the resultant aqueous extract was dried in the oven at 60 °C. The dried extract was kept at 4 °C in the refrigerator for subsequent use (Abdi et al. 2018).

2.3 Synthesis of AgNPs

To synthesize AgNPs, 0.1 g of dried extract from root, stem and leaf were separately dissolved in 10 mL of deionized water. Then, it was mixed with 90 mL of 20 mM silver nitrate solution and incubated under continuous stirring for 90 min at room temperature. UV–visible spectrophotometry was used to monitor the color change during 90 min of incubation and to assess the reduction of Ag⁺ ions (Abdi et al. 2018).

2.4 Determining the Characteristics of AgNPs

The biological reduction of Ag ions with aqueous extract was investigated utilizing UV spectrophotometer at 0-, 20-, 40-, 60-, 80- and 90-min intervals at a wavelength of 420 nm (Jyoti et al. 2016; Abdi et al. 2018). Once the 90 min of incubation was completed, the solution that carried the synthesized nanoparticles was centrifuged three times for 20 min at 12,000 rpm. Afterward, the supernatant was disposed of and the resultant deposit was maintained in the oven at 60 °C for 24 h to dry. Eventually, X-ray diffraction (XRD) [with the scanning 2θ angle ranging from 5° to 80° by step size 0.0260, generator settings: 40 mA, 40 keV, model: X'Pert Pro] was used to detect the formation of crystalline phases of synthesized AgNPs. The crystallinity of the biosynthesized silver nanoparticles was determined using the ratio of the sum of the areas of all peaks in the whole area of the XRD diffractogram and multiplying by 100 (Sari et al. 2015; Ahmed et al. 2016).

To examine the size and shape of the synthesized AgNPs, transmission electron microscopy (TEM) (Zeiss-EM10C-100 kV model) was used. The morphology and size of nanoparticles were also studied using the field emission scanning electron microscopy FE-SEM device

(model SIGMA VP-500) equipped with energy-dispersive spectroscopy (EDS) (Abdi et al. 2018). For investigating functional groups which are present in the biosynthesized nanoparticles and are responsible for the silver ions reduction, Fourier transform infrared (FTIR) spectroscopy was used (Zhang et al. 2016).

2.5 Antibacterial Activity of the Synthesized AgNPs

Antibacterial effect of the biosynthesized AgNPs was investigated by agar well diffusion assay against pathogenic Gram-negative (*Pseudomonas aeruginosa*, *Vibrio harveyi*) and Gram-positive (*Bacillus cereus*, *Staphylococcus aureus*) bacteria (Nanda and Saravanan 2009). These bacteria are important pathogens to aquatic organisms including fish, and controlling them would be a significant practical use for synthesized nanoparticles.

The bacterial pathogens were prepared from the Persian Gulf and Oman Sea Ecological Research Institute, Iran. The plates of nutrient agar medium were prepared, sterilized and solidified. Bacterial cultures were then swabbed on these plates. Three wells of 6 mm diameter were made on Mueller-Hinton agar plate by means of gel puncture. The cultures were swabbed on test media with sterile cotton swab. Before using, 10 mg of AgNPs was added to 1 ml of deionized water, vortexed for 15 min and sonicated for 15 min at 30 °C to prepare the colloidal solution of the AgNPs. Using a micropipette, 20 µl of synthesized particles was loaded on to each of the well, and then, the plates were incubated at 37 °C for 24 h. After incubation, the diameter of the zone of inhibition around each well was measured to the nearest mm to determine the susceptibility of the test organisms (Saravanan and Nanda 2010; Ahmed et al. 2016). The bactericidal activity of the green synthesized AgNPs was also compared with plant extract.

3 Results

In the present study, the mangrove plant *R. mucronata* mediated green synthesis of AgNPs. To this end, the extract of stem, leaf and root of the mangrove was added to 20 mM AgNO₃ solution and incubation was done at room temperature. As a result, Ag^+ was reduced into Ag^0 and AgNPs were fabricated. The first signs of AgNPs formation became evident by conversion in solution color. The solution with leaf, root and stem extract along with silver nitrate solution was bright yellow and light brown at the start of the reaction. The solution turned dark brown after 90 min of incubation. The appearance of a dark brown color in the aqueous extract after the reaction with silver



ions was a clear sign of change of metal ions into silver metal and formation of AgNPs (Fig. 1).

Among different plant parts, the extract of plant leaf showed the maximum nanoparticle production compared with stem and root, which may be attributed to the secondary metabolites such as flavonoids, polyphenols and tannins present in the leaf extract. Based on the absorbance obtained by UV–visible spectrophotometry at 420 nm, the production of AgNPs by the aqueous extract of *R. mucronata* was approved (Fig. 1).

To confirm the crystallinity of AgNPs, XRD analysis was carried out. The crystalline nature of AgNPs was approved considering the diffraction peaks at 2θ values of 38.53, 46.25, 64.76 and 77.43 corresponding to the planes of (111), (200), (220) and (311), respectively, which can be indexed according to the facets of face-centered cubic crystal structure of silver (Abdi et al. 2018; Prakash et al. 2013) (Fig. 2, A). The peaks were compared with the standard powder diffraction card of Joint Committee on Powder Diffraction Standards (JCPDS), silver file No. 04-0783 (Fig. 2, B). Crystallinity (%) of the biosynthesized silver nanoparticles was 88.54.

To find the size and morphology of produced AgNPs through the herbal extract, TEM was used. The analysis of images recorded by TEM showed that the archived nanoparticles were spherical in shape (Fig. 3). The nanoparticles were in a size range between 1 and 80 nm with mean size of 32.44 nm (Fig. 3). The histogram of biosynthesized AgNPs size distribution is presented in Fig. 4. According to this histogram, the number of nanoparticles in the range of 20–25 nm was more than other that of size groups (Fig. 4).

FE-SEM images analysis revealed that the biosynthesized AgNPs ranged from 10 to 19 nm (Fig. 5). According



Fig. 2 XRD pattern of silver nanoparticles synthesized using *R. mucronata* leaf extract A and XRD pattern of standard silver file No. 04-0783 B

to EDS spectrum, elements such as carbon, silver, oxygen and chlorine were present in the bio-nanoparticles derived from the leaf extract of *R. mucronata*. Silver (73.5%) was the major constituent element as shown in Fig. 6.

Functional groups responsible for the reduction of silver ions and acting as coating agents of silver nanoparticles were identified by FTIR. The results yielded peaks at 3371.96, 1585.05, 1378.30, 1057.39, 823.39 and 521.07 (cm⁻¹) (Fig. 7) which, respectively, represent the alcohol absorption frequency O–H, aromatic ring absorption frequency C=C, alkane adsorption frequency CH₂, alcohol and phenol absorption frequency C–O, and 823.39 cm⁻¹ is non-specific and is related to C–H on the aromatic ring but is an off-plate bending type, and the last one (521.07 cm⁻¹) indicates the absorption rate of alkyl halides (alkyl bromide) C–Br (Ibrahim 2015; Syed Ali et al. 2015).



Fig. 1 UV absorbance of silver nanoparticles synthesized using *R. mucronata* extract at 420 nm. 20 mM AgNO₃ solution **a** without plant extract and **b** with plant extract





Fig. 3 TEM images of silver nanoparticles synthesized using R. mucronata leaf extract



Fig. 4 Size distribution histogram of silver nanoparticles synthesized using *R. mucronata* leaf extract



Fig. 5 FE-SEM image of silver nanoparticles synthesized using *R*. *mucronata* leaf extract

Findings from the study of antibacterial properties of *R. mucronata* extract and synthesized AgNPs by well diffusion method are presented in Table 1. According to these results, the Gram-negative bacteria were more resistant

than other strains. The Gram-positive bacterium of *Bacillus cereus* with the highest amount of inhibition zone was the most sensitive, and Gram-negative bacterium of *Pseudomonas aeruginosa* with the lowest inhibition zone was identified as the most resistant bacteria.

4 Discussion

The results obtained in this study specified that the leaf extract of the mangrove plant R. mucronata was able to biologically produce spherical AgNPs which were in the range of 1-80 nm. The color change observed when mixing the silver nitrate solution with the mangrove extract was the result of the reduction of silver ions by the extract of R. mucronata and indicated that AgNPs has been formed. It seems that the bioactive compounds or biomolecules available in the extract reduce the silver metal ions into AgNPs and cause the change of the color (Gopinath et al. 2012; Sinha et al. 2015; Abdi et al. 2018). Actually, the superficial plasmon-induced vibrations are excited with the synthesized AgNPs and the dark brown color of the extract is appeared (Krishnaraj et al. 2010; Singhal et al. 2011). The increase in vibrations induced by surface plasmon can increase the color intensity of the silver nanoparticle solution. The appearance of dark brown color and yellowish brown color was also reported before as clear signs of the reduction of silver ions and the synthesis of silver nanoparticles using the mangrove Avicennia marina (Gnanadesigan et al. 2012; Abdi et al. 2018).

The AgNO₃ solution color which was transparent at the beginning changed to light yellow upon the addition of the plant extract. It was observed that the expansion of the time duration of incubation resulted in the increase in the color intensity of the biosynthesized AgNPs. In the case of leaf extract, the maximum level of OD value (about 3) was monitored just after incubation for 10 min. To compare,





Table 1 Inhibition zone of Ag NPs and Rhizophora mucronata plant extract against Gram-positive and Gram-negative bacteria

Microorganisms	Zone of inhibition of AgNPs (mm)			Zone of inhibition of plant extract (mm)		
	Leaf	Root	Stem	Leaf	Root	Stem
Bacillus cereus	12.33 ± 0.57	15.66 ± 3.05	18.33 ± 0.57	12 ± 1.00	11 ± 1.73	12.33 ± 0.57
Staphylococcus aureus	14 ± 1.73	17 ± 1.73	17.66 ± 1.52	14.66 ± 1.52	13.33 ± 0.57	14 ± 3.46
Vibrio harveyi	10.33 ± 1.52	14 ± 2.00	13.66 ± 0.57	0.00	9.66 ± 1.52	10 ± 3.00
Pseudomonas aeruginosa	7.66 ± 1.52	12.66 ± 2.08	15.33 ± 0.57	9 ± 1.00	1 ± 1.73	8 ± 1.00

Results (shown as mean \pm SD) were obtained from triplicate. Inhibition zone includes diameter of the wells (6 mm)

maximum OD of 3, 1.39 and 0.5 was, respectively, attained after 20 min, 8 h and 8 h of incubation for the leaf extract of *A. marina* (Gnanadesigan et al. 2012; Balakrishnan et al. 2016; Abdi et al. 2018). In this study, the leaf extract of *R. mucronata* produced the maximum amount of Ag nanoparticles compared with stem and root (Fig. 1). The reducing activity of secondary metabolites like terpenoids, polyphenols and tannins appears to be the reason. Incubation time needed for the maximum level of OD value (about 3) by root and stem was about 20 min which may be due to less amount of secondary metabolites present in these organs compared with the leaf. The confirming



results have also been reported by other researchers (Gnanadesigan et al. 2012; Prakash et al. 2013).

In addition to the color change of solution, the formation of AgNPs is also easily detectable by UV–visible spectrophotometry. In fact, when different nanoparticles are generated from their salts, distinctive absorption peaks are formed. AgNPs usually exhibit absorption peak at wavelengths of the visible range between 400 and 500 nm. Reviewing the studies on synthesis of AgNPs by organisms shows that absorption peak of AgNPs occurs at wavelengths of about 410–450 nm (Yugandhar et al. 2015). In the present study, according to the absorbance rate obtained by UV–visible spectrophotometry at 420 nm, the biosynthesis of AgNPs by aqueous extract of *R. mucronata* was confirmed. The biosynthesized AgNPs peak around 420 nm has also been reported by other studies (Zaheer and Rafiuddin 2012; Jyoti et al. 2016).

Given the peaks shown in XRD, the presence of silver nanoparticle crystals with pronounced peaks was confirmed. The reason is that the reducing agents of the extract of *R. mucronata* leaves have stabilized the synthesized nanoparticles. The results of the XRD pattern for silver nanoparticles were in line with the previous studies (Gnanadesigan et al. 2012; Jyoti et al. 2016; Abdi et al. 2018).

According to the TEM and SEM images, the synthesized AgNPs had a size range between 1 and 80 nm (10-19 nm by SEM) with mean size of 32.44 nm and owned the same spherical morphology. The size distribution histogram revealed that nanoparticles in the size range of 20-25 nm were more than other size groups. An average particle size of 17.30 nm for AgNPs prepared from the leaf extract of the mangrove Avicennia marina has been reported (Abdi et al. 2018). In synthesizing AgNPs using three mangroves of Avicennia alba, Sonneratia caseolaris and Sonneratia apetela, nanoparticles possessing a uniform spherical shape and in the size range of 20-40 nm have also been reported (Bakshi et al. 2015). The detected FTIR peaks in this study coincide with the major functional groups in various chemical classes such as terpenoids and polyphenols. These biomolecules have previously been described to own the potential to reduce the silver ions and form the AgNPs (Nabikhan et al. 2010; Prasad and Elumalai 2011; Abdi et al. 2018). A possible reaction path for the biofabrication of the Ag nanoparticles is shown by reactions 1 and 2 as follows:

$$AgNO_3 + H_2O \rightarrow Ag^+ + NO_3^-$$
(1)

 $2Ag^+ + HO-Polyphenols of Extract-OH$

 \rightarrow O=Polyphenols of Extract=O + Ag⁰ (nanoparticles)

AgNPs have many applications in different fields, of which one of the most important is their killing effect on pathogenic bacteria. The high interactive potential of nanoparticles is attributed to their higher surface-to-volume ratio, which in turn increases the antibacterial activity of AgNPs (Rai et al. 2009; Sarkheil et al. 2017). These nanomaterials can be a good alternative for fighting against the pathogenic bacteria with the lowest toxicity. In the present study, the antibacterial activity of synthesized AgNPs was shown against Gram-positive and Gram-negative bacteria. The results showed that the Gram-negative bacteria were more resistant among the selected strains. Due to the presence of outer membranes that enclose the cell walls in Gram-negative bacteria, it seems logical that these bacteria would be less sensitive to the antibacterial effects of AgNPs. Gram-positive bacteria have been reported to be more prone to synthesized AgNPs by marine algae Urospora sp., so that the amount of inhibition zone for Gram-positive bacteria of Bacillus subtilis and Staphylococcus aureus was higher than that of Gram-negative bacteria of Escherichia coli (Suriya et al. 2012). In analyzing the effect of AgNPs synthesized by green algae Enteromorpha flexuosa on different strains of Gram-positive and Gram-negative bacteria, it was reported that the Gram-positive bacteria with the highest amount of inhibition zone and the Gram-negative bacteria with the lowest amount of inhibition zone were the most sensitive and resistant bacteria, respectively (Yousefzadi et al. 2014). Also, a study on Gram-positive bacteria of Bacillus subtilis and Gram-negative bacteria of Kelebsiella planticola indicated that the Gram-negative bacteria with the least amount of inhibition zone were the most resistant bacteria (Vanaja and Annadurai 2013). It was also deducted that the antibacterial effect of the extracts of leaf, stem and root was different. This may be due to the different amounts and/or types of bioactive compounds present in these plant organs. Various bioactive compounds including flavonoids, alkaloids, saponins, tannins and anthraquinones which are present in different amounts in plants extracts are considered to have activity against several pathogens and therefore aid the antimicrobial activities (Sharma et al. 2012; Abdelmoteleb et al. 2018).

5 Conclusion

(2)

Silver nanoparticles were synthesized successfully by means of utilizing different parts of native mangrove *Rhi-zophora mucronata*. To our knowledge, no previous study has reported the biosynthesis of silver nanoparticles from this native mangrove from Iran. The leaf extract of *R. mucronata* generated the maximum nanoparticle



production in comparison with stem and root. UV–visible spectrophotometry confirmed the formation of silver nanoparticles from *R. mucronata* aqueous extract. The biosynthesized silver nanoparticles were crystalline and spherical based on XRD and TEM and according to SEM/ TEM, in a size range between 1 and 80 nm. Silver was the major constituent element, 73.5%, in EDS spectrum. FTIR analysis revealed that different functional groups present in the mangrove extract caused the reduction of silver ions and helped in the formation of nanoparticles in the biosynthesis procedure. The inhibition zone that was formed in the screening test implied the antibacterial activity of AgNPs that was more intense in Gram-positive bacterial pathogens than Gram-negative ones.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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