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Green synthesis of cobalt oxide nanoparticles from *Clitoria ternatea* flower extracts its characterization and biological activities

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Abstract

Cobalt oxide nanoparticles (Co_3O_4-NPs) have numerous applications and presently the green method for the synthesis of nanoparticles is favored over other methods because of their benefits to the environment. In this study, Co_3O_4-NPs were synthesized by using the flower extracts of *Clitoria ternatea* and Cobaltous chloride hexahydrate as a source of cobalt. The synthesized Co_3O_4-NPs were studied by different techniques such as UV-Visible Spectroscopy, Fourier transform (FTIR), X-ray diffraction (XRD), Scanning Electron Microscopy (SEM) and Energy dispersive analysis. Scanning Electron Microscopy images revealed the spherical and irregular structure of Co_3O_4-NPs having the particle size between 13 to 17 nm. X-ray diffraction showed the crystalline nature of cobalt oxide nanoparticles with Face – centered cubic. The prepared Co_3O_4-NPs showed significant antimicrobial activity against *Streptococcus thermophilus, Aspergillus flavus* and *Aspergillus niger.*

Keywords: *Clitoria ternatea;* Cobalt oxide nanoparticles; Surface functionalisation; Antibacterial activity; Antifungal activity

1. Introduction

The nanotechnology utilizes many physical and chemical properties with distinctive properties. Through nanotechnology, we can advance new materials with sizes less than 100nm. These nanomaterials have expansive applications such as nanomedicine, nanoelectronics and energy production [1-9]. Biosynthetic systems use either biological microorganism such as bacteria, fungi and plant extracts have showed to be simple and economic [10-13]. Synthesis of metal oxide nanoparticles through plant mediated green synthesis has captivated number of scientists and researchers from harmful methods because of its ecological safe, biodegradable and harmless production system [14]. Plant based nanoparticles are synthesized from individual origins of plants and are extensively used in multiple applications. Plants made up of discrete phenolic compounds which are very active. These phenolic compounds act a vital role in redox reactions during the nanoparticles formations [15]. Plant based nanoparticles proffers many advantages including readily availability and contains biomolecular compounds such as alkaloids, terpenoids and quinines that ease nanoparticles synthesis [16, 17]. Co_3O_4-NPs have good catalytic activity [18]. For the synthesis of Co_3O_4-NPs, flower extracts of *Clitoria ternatea* has been used. *Clitoria ternatea* commonly known as Asian pigeon wings is a plant species belonging to the family Fabaceae and native to the Indonesian island of Ternate. According to literature survey, the flower extracts of this plant has not been used for the synthesis of Co_3O_4-NPs [19, 20]. The synthesized Co_3O_4-NPs have been characterized by various techniques and antimicrobial activities are evaluated against *Streptococcus thermophilus*, *Aspergillus flavus* and *Aspergillus niger.*

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2. Experimental

2.1. Materials

The chemicals Cobaltous chloride hexahydrate ($CoCl_3.6H_2O$), Sodium hydroxide (NaOH) were purchased from Merck. The flowers of *Clitoria ternatea* was collected from Sathyamangalam, Erode district, Tamil Nadu.

2.2. Preparation of Flower Extracts

The flowers were washed with tap water followed by distilled water and dried under air at room temperature for 7 days. Then these dried flowers (15g) were taken in a glass beaker and distilled water was added and the mixture was stirred for 3 hours. Then the stirring was stopped and the solution was filtered and stored in a refrigerator for further use.

2.3. Synthesis of Co_3O_4-NPs

Co_3O_4-NPs were synthesized by the plant-mediated green synthesis method in which distinct functional groups present in plants acts as a reducing agent and results in the formation of nanoparticles. 15ml of aqueous flower extracts was taken in a beaker and 25ml of 1M aqueous solution of $CoCl_3.6H_2O$ was added to it. Then placed it on a magnetic stirrer for 20 min, after 20 min NaOH was added drop wise and colour change was observed. The resultant extract solution was centrifuged at 7000 rpm for 10 min, and then the supernant was discarded and the pellet containing precipitate of Co_3O_4-NPs was collected and washed with distilled water to remove impurities and transferred into a china dish. The precipitates were kept in an oven at 50°C for drying. The dried fine powder used for the characterization.

2.4. Characterization

The Co_3O_4-NPs were characterized by various techniques. The absorption spectra of the Co_3O_4-NPs were studied using UV-Visible spectroscopy. Surface functionalisation of *Clitoria ternatea* flower extracts on the surface of Co_3O_4-NPs were studied by FTIR (Schimadzu IR Affinity model 1s double beam spectrometer) with the wave number range of 4000 cm^{-1} to 400 cm^{-1}. The crystalline nature and phase identification were studied by XRD (Empyrean, Malvern Panalytical) method with CuKα (λ=1.54 A°) radiation. Surface morphology and particle size of the prepared nanoparticles were analyzed by Scanning electron microscopy technique (Carl Zeiss (USA)-model-Sigma with Gemini column). EDX performed to know about the estimation of the elemental composition of Co_3O_4-NPs.

2.5. Anti-Bacterial activity Analysis

Petri plates containing 20 ml nutrient agar medium were seeded with 24 hr culture of bacterial strains were adjusted to 0.5 OD value according to McFarland standard, (*Streptococcus thermophilus)*Wells were cut and concentration of Co_3O_4-NPs (500, 250, 100 and 50 μg/ml) was added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Gentamicin antibiotic was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA).

2.6. Anti-Fungal activity Analysis

Petri plates containing 20 ml potato dextrose agar medium were seeded with 72 hr culture of fungal strains (*Aspergillus flavus* and *Aspergillus niger)*wells were cut and concentration of Co_3O_4-NPs (500, 250, 100 and 50 μg/ml) was added. The plates were then incubated at 28°C for 72 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Amphotericin B was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA).

3. Results and Discussion

3.1. UV-Visible spectroscopy

The flower extract of *Clitoria ternatea* 1(a) shows an absorption band around 255-260nm which corresponds to the presence of alkaloids, flavonoids, phenolic compounds, amino acids and terpenoids. The UV-Visible spectrum of Co_3O_4-NPs 1(b) revealed a strong absorption in visible region around 200-350nm [38].

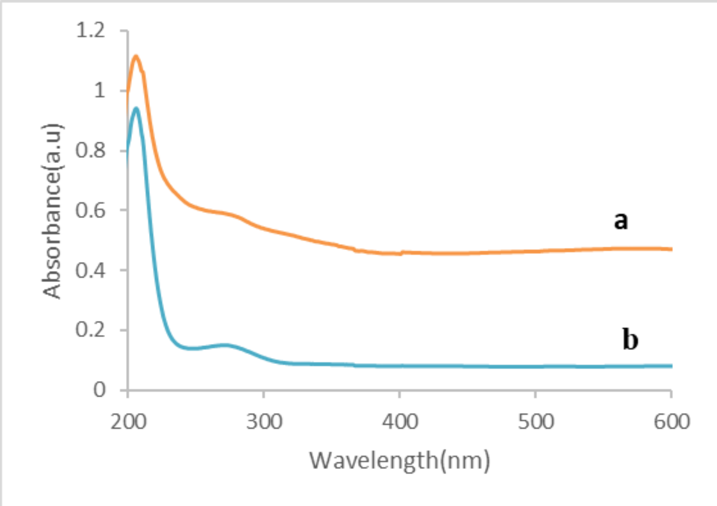

Figure 1 UV-Visible spectrum of *Clitoria ternatea* flower extract 1(a) andCo_3O_4-NPs synthesized with *Clitoria ternatea* flower extract 1(b)

The Co_3O_4-NPs synthesized from the flower extract 1(b) shows strong absorption bands at 288nm, confirms the formation of Cobalt oxide nanoparticles and particles were stable.

3.2. FT-IR

The FT-IR transmission spectra of *Clitoria ternatea* flower extract and Co_3O_4-NPs synthesized from the flower extract were shown in figure 2.

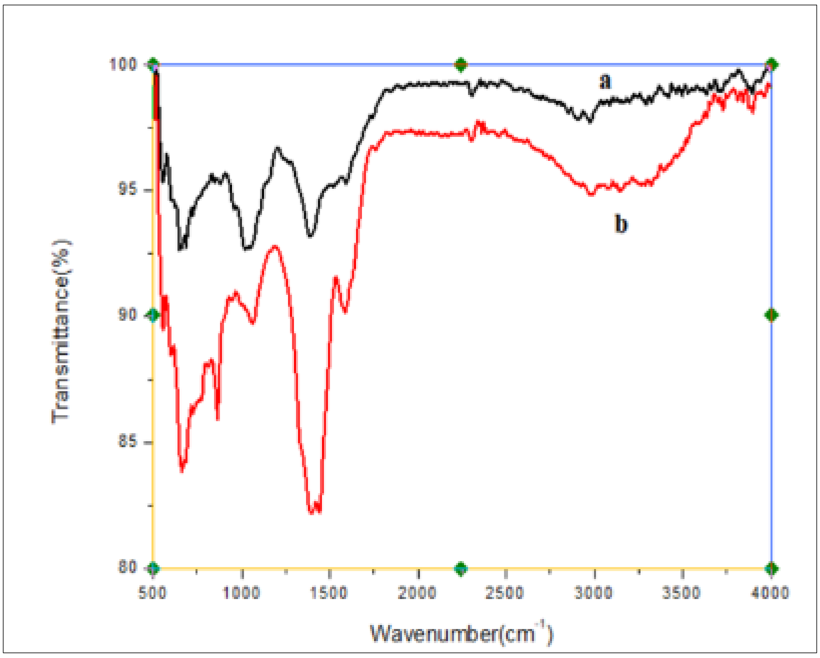

Figure 2 FT-IR spectra of *Clitoria ternatea* flower extract 2(a) and Co_3O_4-NPs synthesized from the flower extract of *Clitoria ternatea* 2(b)

The FTIR spectra of flower extract *Clitoria ternatea* shows peak at 3417cm^{-1},2978 cm^{-1}, 1743 cm^{-1} which shows the presence of hydroxyl group, carbonyl group, C-O of phenols and C-H groups respectively. The spectrum was examined for the biomolecules responsible for the stabilization of Cobalt oxide nanoparticles. The FTIR spectra of green Co_3O_4-NPs shows strong peaks at the wavelength of 3842 and 3726 cm^{-1} corresponds to O-H stretching vibration of phenolic compounds [21]. The peaks at 2978cm^{-1} were the vibrations of lipids present in the flower extract of *Clitoria ternatea* [22]. The peak at 1743cm^{-1} is due to C=O stretching of polysaccharides [23].

3.3. XRD

XRD analysis was performed to confirm the synthesis of Co_3O_4-NPs. To know the crystalline structure XRD analysis was carried out and major peaks were observed at Braggs angles 16.8°,30.1°,36.4°,43.8°,54° and 60.1° corresponded to the lattice planes of (111),(220),(311),(400),(511) and (440) respectively that could be indexed to the face centered-cubic phase of Co_3O_4-NPs in the previously reported literature data [24-32].

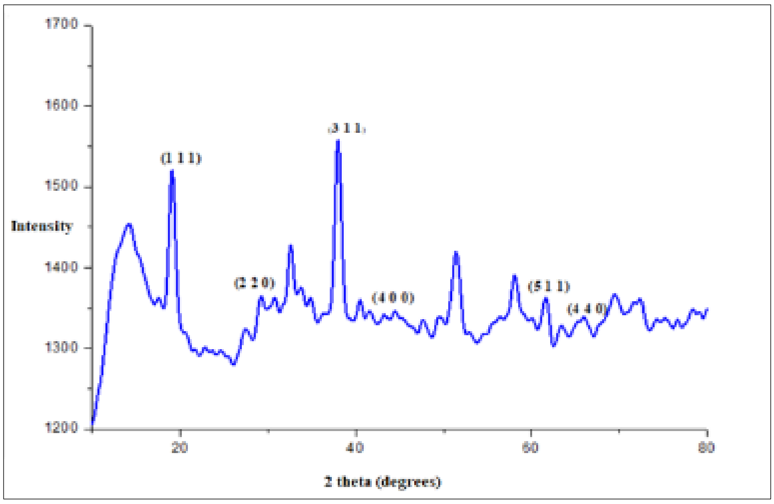

Figure 3 XRD image of Co_3O_4-NPs synthesized from the flower extract of *Clitoria ternatea*

3.4. SEM

The morphology of Co_3O_4-NPs was determined using Scanning electron microscope. The SEM images of Co_3O_4-NPs shows that spherical shaped nanoparticle with diameters ranging from 13-17 nm.

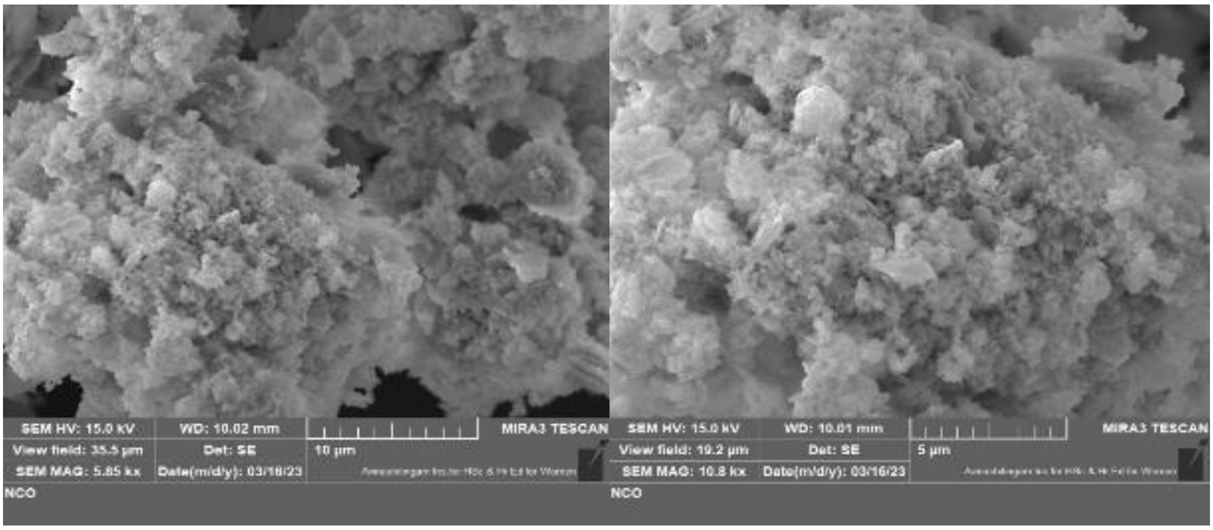

Figure 4 SEM image of Co_3O_4-NPs synthesized from the flower extract of *Clitoria ternatea* at different magnifications

The surface of as synthesized nanoparticles is very smooth, which carries out better contact with the bacterial cell wall and hence increases bacterial killing ability of nanoparticles. Such behaviour of smooth surfaced nanoparticles has already been established in the literature [33].

3.5. EDX

The elemental composition of synthesized Co_3O_4-NPs synthesized from the flower extract of *Clitoria ternatea* was studied from EDX analysis figure5. In this figure, major peaks indicate the Co and O of the synthesized NPs. However, some minor peaks of sodium, carbon and chlorine are also present which are attributed to the flower extract used. The elemental composition of the nanoparticles shows 18 weight per cent cobalt and 39 weight per cent oxygen corresponding to Cobalt oxide (Co_3O_4).

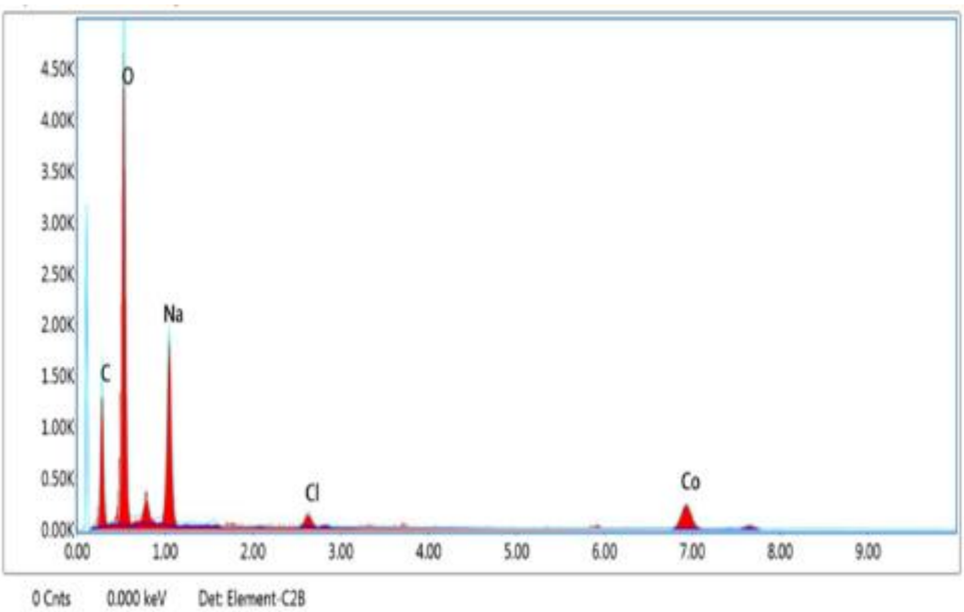

Figure 5 EDX image of Co_3O_4-NPs synthesized from the flower extract of *Clitoria ternatea*

3.6. Anti-bacterial activity

Antimicrobial and anti-bacterial activities of Co_3O_4-NPs are presented in Table 1. Cobalt oxide nanoparticles produced good anti-bacterial activity against *Streptococcus thermophilus* [34-35]. The gram-positive bacteria *Streptococcus thermophilus* showed maximum zone of inhibition (14$\pm$0.7).

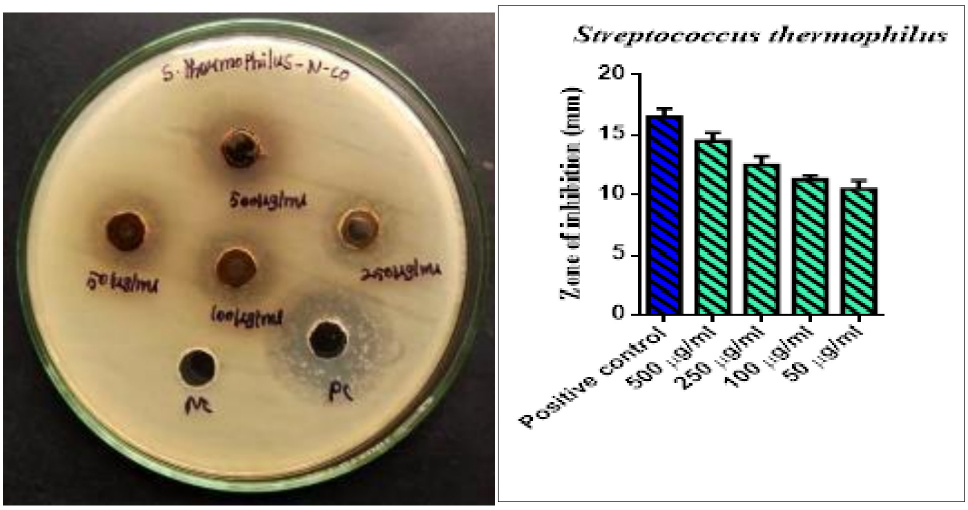

Figure 6 Effect of sample Co_3O_4-NPs synthesized from the flower extract of *Clitoria ternatea* against *Streptococcus thermophilus*

Table 1 SD± Means of zone of inhibition obtained by sample Co_3O_4-NPs synthesized from the flower extract of *Clitoria ternatea* against *Streptococcus thermophilus*

S. No	Name of the test organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				
			500 µg/ml	250µg/ml	100 µg/ml	50 µg/ml	PC
1.	*Streptococcus thermophilus*	Co_3O_4-NPs	14.5±0.7	12.5±0.7	11.25±0.7	10.5±0.7	16.5±0.7

3.7. Anti-fungal activity

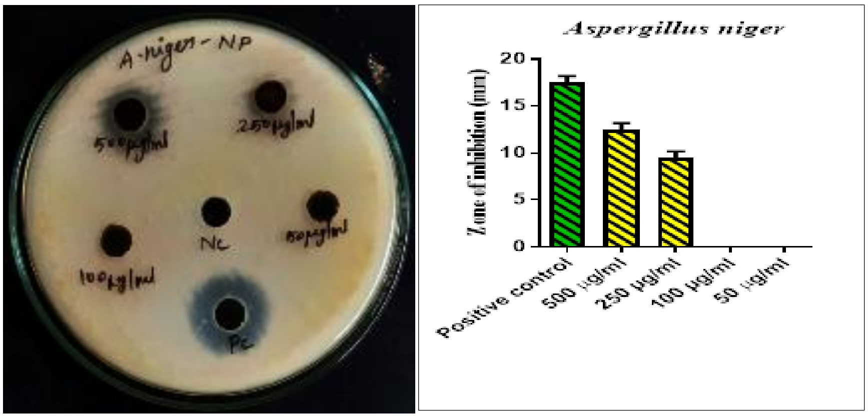

Figure 7 Effect of sample Co_3O_4-NPs synthesized from the flower extract of *Clitoria ternatea* against *Aspergillus niger*

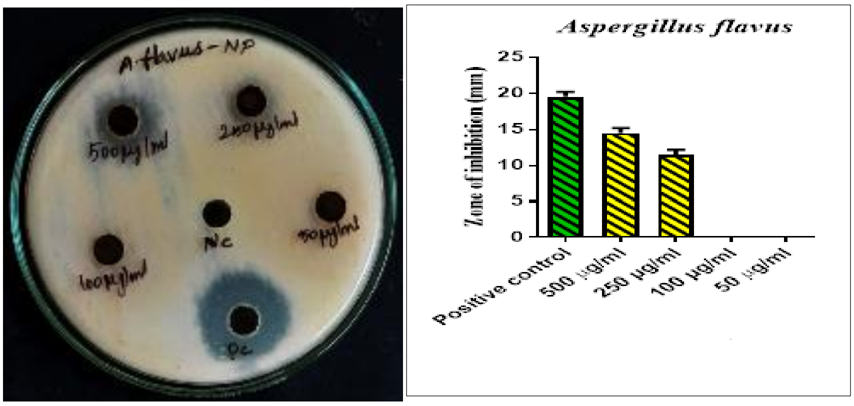

Figure 8 Effect of sample Co_3O_4-NPs synthesized from the flower extract of *Clitoria ternatea* against *Aspergillus flavus.*

Table 2 SD± Means of zone of inhibition obtained by sample NP against *Aspergillus flavus* and *Aspergillus niger.*

S.No	Name of the test organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1.	*Aspergillus niger*	Co_3O_4-NPs	12.5±0.7	9.5±0.7	0	0	17.5±0.7
2.	*Aspergillus flavus*		14.5±0.7	11.5±0.7	0	0	19.5±0.7

Antifungal activities of Co_3O_4-NPs are presented in Table2. Cobalt oxide nanoparticles produced good anti-fungal activity against *Aspergillus flavus* and *Aspergillus niger* [36-37]. Among both classes of fungal *Aspergillus flavus* and *Aspergillus niger*, the maximum zone inhibition showed by *Aspergillus flavus* (14$\pm$0.7).

4. Conclusion

Cobalt oxide nanoparticles were synthesized by cost effective, biodegradable and ecological friendly green method by using flower extract of *Clitoria ternatea.* The prepared nanoparticles were analyzed by UV-Visible spectroscopy, FTIR, XRD and SEM techniques. These techniques showed successful synthesis of Cobalt oxide nanoparticles. Anti-bacterial activity of synthesized Cobalt oxide nanoparticles was analyzed against gram positive bacteria *Streptococcus thermophilus.* The Anti-fungal activity of synthesized Cobalt oxide nanoparticles was analyzed against *Aspergillus flavus* and *Aspergillus niger.* It was found that by increasing concentration of Cobalt oxide nanoparticles, anti-bacterial activity and Anti-fungal activity was increased.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no competing interests.

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