



Green Synthesis and Characterization of Spherical Structure Silver Nanoparticles Using Wheatgrass Extract

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Abstract

In this research, silver nanoparticles were successfully synthesized using green approaches. Biosynthesis using plant extract was applied as a green method to the preparation of silver nanoparticles. In this work, the effect of *Wheatgrass* extract was investigated as reducing agent. As-synthesized silver nanoparticles were characterized using several physical methods such as, powder X-ray diffraction (XRD), Transmission electron microscopy (TEM), FT-IR analysis, UV-vis spectroscopy and dynamic light scattering method (DLS). The result of TEM images showed that silver nanoparticles were formed as spherical particles with high monodispersity and size 21-32 nm. Additionally, the size distribution of these nanoparticles was calculated with DLS histogram which the result was in agreement with the result of TEM image with average size 28 nm. Since, the *Wheatgrass* extract play an important role as the capping agent, the present of this extract on the surface of silver nanoparticles was to study using FTIR analysis and the result approved the present of many functional groups on the surface of nanoparticles. Briefly, this strategy provides a simple, cost effect and eco-friendly way to prepare nanoparticles without using hazardous chemical agents.

Keywords: Silver nanoparticles, Green synthesis, Biosynthesis, *Wheatgrass* extract

1 Introduction

Environmental engineering is an important field of study to increase the quality of human life. Nanotechnology has a wide range of applications in environmental engineering. Over the last few decades, green eco-friendly strategy has remarkably been considered for the synthesis of a wide range of nanostructures (1). Many types of nanostructures, such as metal nanostructures (2), magnetic nanoparticles (3), nanocomposite (4) and metal oxide nanoparticles (5), were synthesized using this method. The synthesis of nanomaterials by conventional methods have a fundamental challenge during synthesis procedures due to applying chemical substrate as the capping agent, surfactant

and reducing the agent (6,7). These challenges include the introduction of harmful contaminations into an environment as well as the presence of impurities (such as organic and inorganic materials) in final nanostructures. On the other hand, instead of chemical and physical methods, the replacement of green synthesis approaches is due to the advantages of this method, which includes simple, eco-friendly, inexpensive and availability (2). The green synthesis of the nanomaterials was carried out using the types of microorganism (8), plant (9), different fruit extracts (10) and biodegradable polymers (11). These natural agents greatly affect the particle size, stability and morphology of nanostructures (11).

Nowadays, metal nanoparticles (MNPs) have dramatically attracted many researchers because of physicochemical properties and also an application in various fields (12,13). The size of nanoparticles are between 3 to 100 nm that play a major role in properties and application of this material (14). Beside, large surface to volume ratio of nanoparticles has a significant effect on its activity and performance in all of applications (15,16).

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Some applications of these structures involve industrial (17), agricultural (18), water treatment (19) and medicine (20) and also these materials were used as computer transistors (21), medical imaging agent (22), chemical sensors (23) and filters (24).

Noble metal nanostructures such as gold (Au) (25), silver (Ag) (26) and platinum (Pt) (27) were known as main nanoparticles in nanotechnology and nanomedicine fields because of surface plasmonic resonance (SPR) properties (28). In recent years, Ag NP has been considered in comparison with others due to its unique properties. This material was employed in various application as catalyst/photo-catalyst in combination with other materials (29,30), sensor/biosensor (31) and antimicrobial agent (32). According to previous literatures, silver nanoparticle can be an inhibitory effect on several types of gram positive and gram negative bacteria such as *aureus*, *Syphilis*, *Escherichia coli*, *Vibria cholera*, *Bacillus subtilis*, *Staphylococcus typhus* and *Pseudomonas aeruginosa*. Also, Ag NP was used in several drugs form such as topical ointments as antibacterial agent (33,34). Exceptional properties of Ag NPs are strongly related to the synthesis methods and strategies. Some physical and chemical approaches were employed for the preparation of silver nanoparticles using chemical agent and device. These methods included photochemical reduction (35), evaporation- condensation (36), laser ablation (37), electrochemical techniques (38), chemical reaction by organic and inorganic agents, gamma irradiation (39), thermal decomposition of silver oxide in water and ethylen glycol (15), microwave processing (40). All of chemical methods need to hazardous and toxic agent such as the surfactant and capping agent as well as in physical method need to high energy (such as microwave and UV irradiation) and complex device for synthesis of Ag NPs. Indeed, using these methods can be a threat to the health of the environment and also contrary to the principles of green chemistry. In this regard, applying microorganism and

plant was developed as the green agent for the synthesis of silver nanoparticles (41,42). Additionally, some types of natural agents, such as fungus (43) and enzymes (43), were considered by researchers, while plant and plant extract has widely been progressed as the agent to achieve this aim. Plant extract can be played not only the capping agent but also the surfactant role in this synthesis method. In addition, the plant extract is a non-toxic and safe substitute for hazardous chemical reducing the agents in green synthesis of Ag nanoparticles (2). In previous literatures, several types of plant were reported such as *Bamboo charcoal* (44), *marigold flower* (45), *Aloe vera* (46), *Tamarind fruiti* (47), *Diospyros paniculata* (48), *Azadirachta indicia* (49) and *Artocarpus heterophyllus* (50).

Wheatgrass plant was also known with other names such as *Agropyron repens*, *Brote del Trigo*, *Agropyre*, *Doggrass*, *Elymus repens*, *Graminis Rhizoma*, *Quackgrass*, *Scotch Quelch*, *Triticum repens* and *Wheat Grass*. This plant is formed in young grass of the common wheat plant that is usually called as *triticum aestivum*. *Wheatgrass* has many pharmaceutical properties as juice and powder for both humans and animals (51). Some medicines properties of this plant involve reducing high blood pressure and cholesterol, preventing tooth decay, heal wounds and antibacterial. Extract *wheatgrass* was composed of a variety of compounds such as vitamin C, vitamin E, thiamin, niacin, pantothenic acid, protein, riboflavin, polyphenol, amino acid and so on (52). Although several studies have been carried out on syntheses of nanoparticles (53–65), in this research, silver nanoparticle has been synthesized by the green strategy applying extract *wheatgrass* in mild condition. Aqueous *wheatgrass* solution provides a safe, cost effect and environment friendly approach for the green synthesis of Ag NPs in water media. Additionally, the final product was characterized by many types of the physical method.

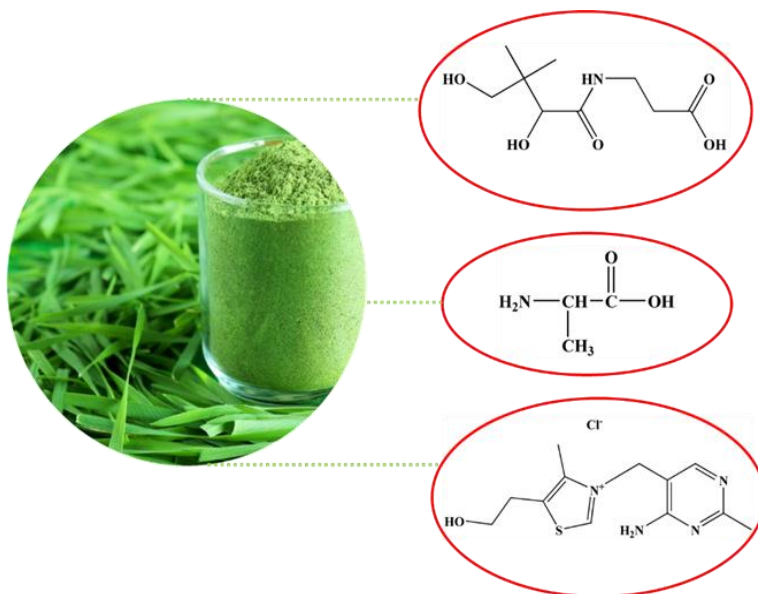


Fig. 1. Display of the important molecular structure in *wheatgrass* extract

2 Material and Method

2.1 Instruments

The powder X-ray diffraction (XRD) pattern measurements of the samples were recorded on a Holland-Philips X-ray powder diffractometer using Cu K α radiation ($\lambda = 0.1542$ nm) with scattering angles (2θ) of 5-80°, operating at 40 kV and a cathode current of 20 mA. Additionally, some specimens of synthesized Ag NPs for TEM studies were prepared by ultrasonic dispersion of the NPs in ethanol, and the suspensions were dropped onto a carbon-coated copper grid. TEM was carried out using a (CM30 3000Kv). FT-IR spectra were recorded to investigate the functional group on samples which carried out on a Bruker VERTEX 80 v model using the KBr disk method. The size distribution of Ag NPs was characterized by the DLS approach, using a computerized inspection system (MALVERN Zen3600) with DTS® (nano) software. UV-Vis spectroscopy (UV-Vis) analyses were taken using a Varian Cary 50 UV-vis spectrophotometer. Spectra were recorded in a range of 350-800 nm.

2.2 Materials

Silver nitrate was purchased from Merck Company for this study and was used without any renewed purification. Fresh samples of *Wheatgrass* parts consisting of leaves and stems were identified and collected. To remove pollution, all of glassware were cleaned with dilute HNO₃ acid and rinsed with distilled water as well as dried in an oven.

2.2.1 Preparation of Wheatgrass Extract

It is preferable that fresh Wheatgrass extract be used to reduce Ag⁺ to Ag⁰. The plant was collected from its growing area and then it was washed with distilled water to remove any pollution and dried in the dark place. The dried plant was grinded to obtain powders with mesh 20 for extract using Soxhlet system. Hence, 5 g powder plant was

added to distilled water and transferred to Soxhlet system for the extract and then the fresh extract was cooled at room temperature to use in next step.

2.2.2 Synthesis of silver nanoparticles

At first, 20 ml different concentrations of Wheatgrass extract from 5% to 20% was prepared and also 5 ml an aqueous solution from Ag NO₃ (0.01mM,) prepared for per synthesis of Ag NPs in separately beaker. In each test, 20 ml of fresh Wheatgrass extract was added to 5 ml AgNO₃ (0.01mM) in round-bottom flask and stirred with magnetic stirrer under reflux condition for 30 min which after this time, the color of suspension changed from light- yellow to dark-brown. Specifically, changing the color in synthesis of Ag nanoparticle indicated that the Ag⁺ reduced to Ag⁰ in the presence of plant extracts. After this, suspension was cooled at room temperature and stocked to characterize.

3 Result and Discussion

Ag NPs was prepared by the reaction of AgNO₃ while Wheatgrass extract was used as solvent and the capping agent. Therefore, in the first step, crystal structure of Ag NPs was determined by X-ray diffraction analyses which the results of this analysis were indicated in figure 2. The diffraction peaks are in agreement with a face-centered cubic (FCC) phase that this list peaks matches the reference file with JCPD= 04-0873. The spectrum displays five diffraction peaks at $2\theta = 38.22, 44.37, 64.54$ and 77.47 represent as well as this peaks related to (111), (200), (220) and (311) plane of Ag NPs with FCC crystalline structure. Additionally, the unassigned predominant sharp peak in $2\theta = 32.40$ and unassigned weak peaks in $2\theta = 28, 46$ and 65 , which are marked with the mark (#), correspond to crystalline phase of bioorganic in Wheatgrass extract on surface of Ag NPs (48,66,67).

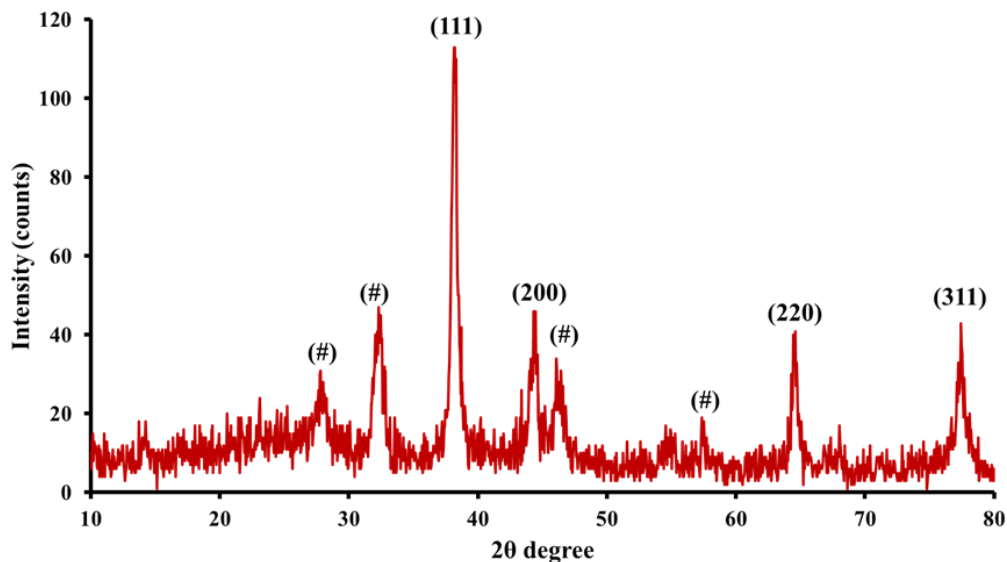


Fig. 2. XRD pattern of biosynthesized Ag NPs using *wheatgrass* extract.

Figure 3 show the comparison of UV-visible absorption spectroscopy diagram of Wheatgrass extract, AgNO_3 solution and Ag NPs. According to the results, colorless AgNO_3 solution hasn't any obvious peak at 350-800 nm range and light-yellow Wheatgrass extract has a weak peak at 410 nm. While the reaction of colorless AgNO_3 salt solution with light-yellow Wheatgrass extract solution led to formation a dark-brown suspension which this suspension has a sharp peak about 450 nm at 350-800 nm range. Therefore, changing the color and subsequently the appearance of the peak in visible range confirm the success synthesis of Ag NPs by this green method which this observed peak is compatible with the previous report at 400-500 nm range (50).

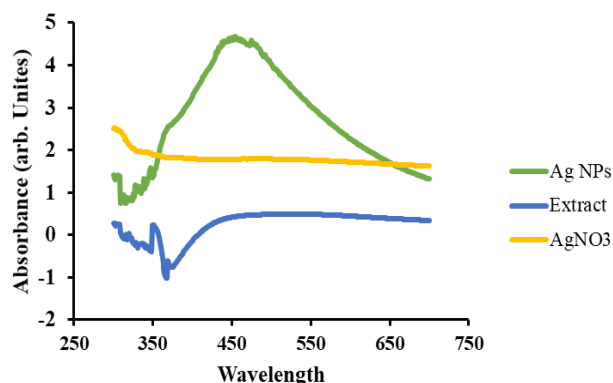


Fig. 3: UV-vis spectroscopy of AgNO_3 wheatgrass extract and Ag NPs.

Moreover, FTIR analysis was carried out to approve the formation of Ag NPs at 4000-500 cm^{-1} range. Figure 4 indicates many peaks which these peaks related to functional groups of Wheatgrass extract on surface of Ag nanoparticles. According to figure 4, three peaks at 3500 to 3400 cm^{-1} range are corresponded to N-H stretching of the amide group and observed band at 3232 cm^{-1} is assigned to O-H stretching of the pantothenic acid group. In addition, the very weak band of alkane C-H stretching vibrations of methyl, methylene, and methoxy groups can be observed at 2922 cm^{-1} and also the binary peak at 1632 and 1616 can be assigned to C=O stretching vibrations of pantothenic acid. Moreover, the peak at 1383 represented the C-H bending and peak in 1111 could be related to C-OH bond stretching. Finally, the vibration of aromatic ring in Wheatgrass extract can be seen at 616 cm^{-1} . The result of the FTIR analysis approved the capping agent role of the extract in the formation of Ag NPs which scheme 1 showed this role of the extract in the formation and growth of nanoparticles.

As a general trend, the low and high-magnification TEM image was carried out to investigate the morphology and size distribution of as-synthesized silver nanoparticles. High-magnification TEM image in figure 5a reveals that Ag NPs were formed as a spherical particle with high high-quality crystalline structure. The images display the

monodispersity in all of the particles without any aggregation which this concept demonstrates the successful synthesis of silver nanoparticles in the presence of plant extracts as the capping agent and surfactant to control the morphology and size of nanoparticles. The size distribution of spherical nanoparticles was considered about 26-30 nm in diameter. Fundamentally, monodispersity, small size and spherical structure for Ag nanoparticles are three important factors in medicine applications. Hence, table 1 compares the used plant and the particle size of the silver nanoparticles in the previous work with Ag nanoparticles synthesized in this work.

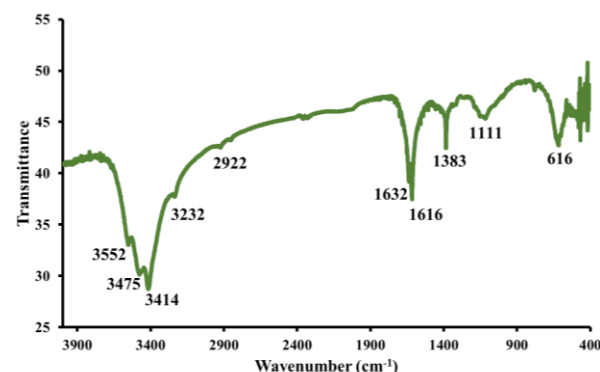


Fig.4: FTIR spectroscopy of prepared silver nanoparticles by green synthesis.

Additional information on the particle size distribution was obtained using DLS analysis. DLS size distribution histogram of as-prepared Ag NPs was illustrated in figure 6. The results show that size distribution ranges of biosynthesized Ag NPs are from 21 to 36 nm which this range matches with the result of TEM image. The average particle size distribution was calculated that this value was 28 nm for Ag NPs. Narrow DLS size distribution histogram in this case is approved the monodispersity of silver nanoparticle and the main role of Wheatgrass extract as the capping agent.

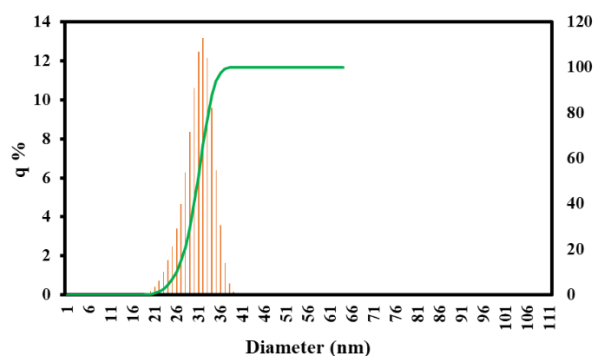
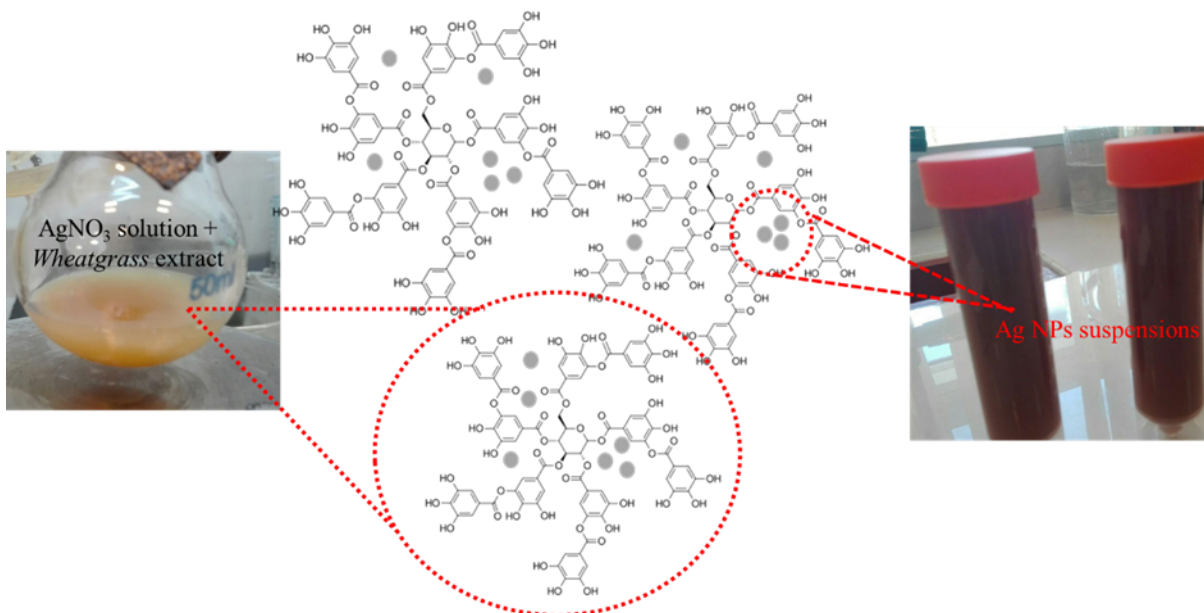


Fig. 6: DLS of green synthesized Ag NPs by wheatgrass extract



Scheme 1. The role of polyphenolic structures as the capping agent to control of Ag^0 crystal growth

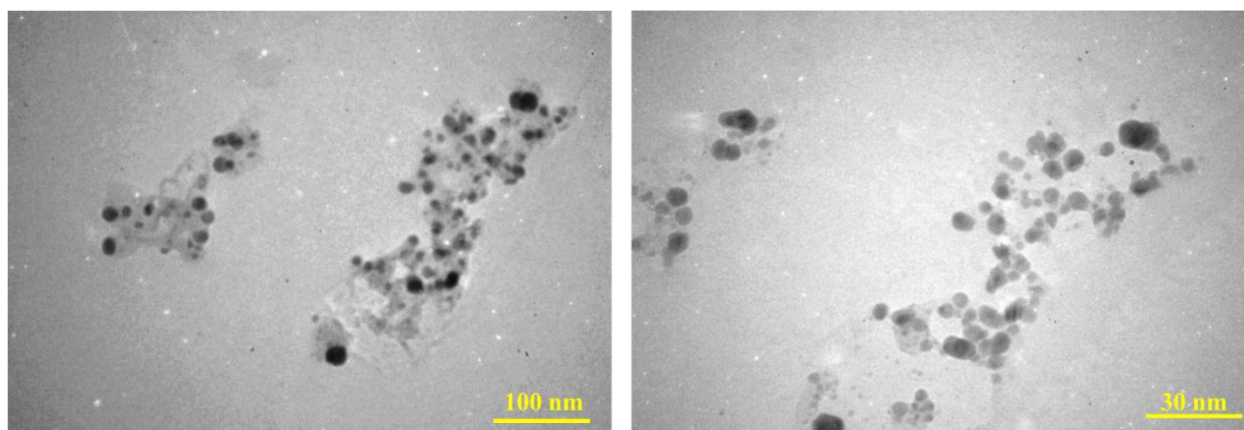


Fig. 5: TEM image of synthesized AgNPs using wheatgrass extract (a) high-magnification and (b) low magnification.

Table 1: Deference plant for biosynthesis of silver nanoparticle and their size and morphology compared to prepared silver nanoparticle in this study.

Plant	Type of Nanoparticle	Size (nm)	Morphology	References
Catharanthus roseus	silver	35-55 nm	Spherical	(42)
Tamarind fruit	silver	200 nm	Triangles, Pentagons and Hexagons	(68)
Azadirachta indica	silver	34 nm	Spherical	(49)
Prunus japonica (Rosaceae)	silver	24 nm	Spherical	(69)
Azadirachta indica (neem)	gold, silver & silver-gold alloys	5-35 & 50-100	Spherical, Triangular, Hexagonal	(50)
Carica papaya	silver	60-80	spherical	(70)
Agropyron repens	silver	21-39	spherical	This work

4 Conclusion

Green synthesis of nanomaterials is one of the very interesting topics in nanotechnology field. In this regard, biosynthesis using the plant and plant extract was progressed in recent years. In this work, the Wheatgrass extract was applied as reducing the agent to convert the Ag^+ cation in AgNO_3 solution to Ag^0 . Thus, the reaction of

AgNO_3 solution in the presence of the Wheatgrass extract led to preparing Ag NPs. Different characterization methods, such as XRD, TEM, FTIR, DLS and UV-vis spectroscopy, confirmed the successful synthesis of Ag NPs. The result of TEM image and DLS analysis were in agreement and showed average particle size 28 nm. Similarly, the spherical structures for Ag NPs were

indicated by TEM images. Additionally, FTIR results displayed that Wheatgrass extract was as the capping agent and surfactant to control of morphology and particle size of these nanoparticles. Finally, this method can be employed to synthesis any types of metal nanoparticles in large scale as well as remove many toxic chemical reagents for the preparation of nanomaterials.

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Ethical issue

Authors are aware of, and comply with, best practice in publication ethics specifically with regard to authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests and compliance with policies on research ethics. Authors adhere to publication requirements that submitted work is original and has not been published elsewhere in any language.

Competing interests

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Authors' contribution

All authors of this study have a complete contribution for data collection, data analyses and manuscript writing.

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