Asian Journal of Chemistry; Vol. 31, No. 9 (2019), 2037-2041

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2019.22085

Plant Mediated Green Synthesis, Catalytic Activity and Antibacterial Assay of Silver Nanoparticles

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Received: 18 March 2019; *Accepted*: 23 April 2019; *Published online*: 31 July 2019; AJC-19494

Plant mediated green synthesis of silver nanoparticles (AgNPs) was carried out with the plant extract of *Vitex negundo* Linn., a simple method without using harmful chemicals. Herein, a root bark extract is used as reducing and capping agent for synthesizing AgNPs from silver nitrate solution. The characterization of AgNPs were obtained by UV-visible spectrophotometer, X-ray diffraction, Fourier transform infrared spectroscopy, scanning electron microscopy and energy dispersive spectroscopy. The results were confirmed the presence of silver nanoparticles with UV-visible absorption peak at around 433-443 nm and its crystalline nature by XRD analysis. The FTIR spectrum confirmed that the synthesized AgNPs are stabilized and protected by various functional groups of biomolecules in extract. The SEM morphology showed the triangular or spherical shape of AgNPs with the size in the range of 80-100 nm and EDS confirmed the presence of elemental silver in the sample. The catalytic activity of AgNPs was investigated in the synthesis of 5-substituted 1*H*-tetrazole. Furthermore, antibacterial profile showed that biopotent AgNPs exhibited moderate to good antibacterial activity.

Keywords: Green synthesis, *Vitex negundo* L., Silver nanoparticles, Catalytic activity, Antimicrobial activity.

INTRODUCTION

Nanotechnology considered that the nanoparticles acting as bridge in between atomic structures and bulk size materials [1]. In recent years, noble metal nanoparticles such as gold, platinum and silver has been extensively studied due to their unique size dependent properties, large specific surface area and their important biological applications in the scientific fields of bioengineering, optoelectronics, catalysis and biosensing [2]. Among these noble metal nanoparticles, silver is the most important nanomaterials for the researchers in which 500 tons of silver nanoparticles (AgNPs) were produced per year and is estimated to grow in the upcoming years [3]. It has been acknowledged that silver nanoparticles have gained considerable attention for their unique nature and high performance in the different scientific fields like solar energy, optical receptors [4,5], energy storage devices, batteries, catalysis in chemical synthesis [6], food processing industry [7], bio-labeling and antibacterial agents [8,9]. In ancient times, silver metal is used to treat open wound infections and chronic ulcers and in particular, silver nanoparticles are excellent antimicrobial agents and catalysts in many chemical reactions [10].

Because of huge applications in various fields, metallic nanoparticles can be synthesized by physical, chemical and biological methods. Physical and chemical approaches of synthesis includes laser ablation, pyrolysis, decomposition, electrochemical methods, microwave assisted techniques, sol-gel and wet chemical procedures [11,12]. However, those expensive methods have toxic impact on human health, which restricts their applications in many areas [13,14]. Hence, there is an urgent need to promote green eco friendly processes in chemistry and its technologies to minimize the generation of hazardous and toxic materials to establish a sustainable process [15]. Recently, green methods of synthesis using renewable materials, non-toxic chemicals, environmentally benign solvents are used to synthesize metallic nanoparticles [16,17]. So, these biological methods of nanoparticle synthesis are devoted as eco friendly green method when compared to the other physical and chemical approaches.

Bio-molecules from several biological resources are used to synthesize metallic nanoparticles by biological methods. It is well known that the extracts derived from various parts of plant consists of different biomolecules will act as reducing and capping agents in green synthesis of metallic nanoparticles in a variety of shapes and sizes. This method of biosynthesis is a cost effective for large scale production which consists of utilization of environmentally benign plant extracts which contain complex biomolecules [18]. Therefore, in this green method of synthesis different types of non-toxic herbal chemicals like flavones, terpenes, aldehydes, ketones, carboxylic acids and amides are directly involved in the reduction of silver ions into silver nanoparticles [19].

Heterocyclic compounds were bagged great importance in the form of pharmaceuticals, agrochemicals, additives and modifiers due to their biological properties [20]. Researchers continuously searching for new synthetic protocols for the synthesis of heterocyclic compounds and are succeeded with the field of nano catalysis which involves the use of metallic nanoparticles as catalysts under various conditions [21]. Tetrazoles are five membered heterocyclic compounds which contain four nitrogen atoms and one carbon. It is an isosteric replacement for carboxylic acids having a wide scope of biological and commercial applications [22,23]. In concern with its wide applications, several methods of synthesis of tetrazoles are well documented in the literature. Silver nanoparticles synthesized by biological method are used as catalysts for the synthesis of tetrazole analogs [24,25].

Vitex negundo commonly called as Chinese chaste tree or five leaved chaste tree or Monk′s pepper′ belongs to Verbenaceae family. This aromatic shrub is source for flavonoids, flavones, glycosides, volatile oil, tri terpenes, tannins and many others [26]. Though, all parts of *Vitex negundo* are used in the indigenous system of medicine, the leaves are the most potent for medicinal use. The roots of plant are long woody, cylindrical, tortuous with grey brown colour possessing medicinal values of tonic, febrifuge, diuretic, expectorant antirheumatic and useful as a demulcent in dysentery, colic, incephalalgia, uropathy, otalgia, wound and ulcers. The extract of root bark is extensively used as a source of herbal medicine for the presence of medicinal phytochemicals [27-29]. Considering the importance of metal nanoparticle synthesis using different plant extracts, the present study is aimed at synthesizing the AgNPs by *Vitex negundo* root bark extract at room temperature without using toxic chemicals. The method applied for synthesis is simple, rapid and environmentally benign. After going through the literature survey, as per the authors′ knowledge *Vitex negundo* has not yet been utilized for the synthesis of silver nanoparticles. In addition, we report the catalytic utility of silver nanoparticles for the synthesis of 5-substituted 1*H*-tetrazoles and antibacterial activity against some bacterial strains.

EXPERIMENTAL

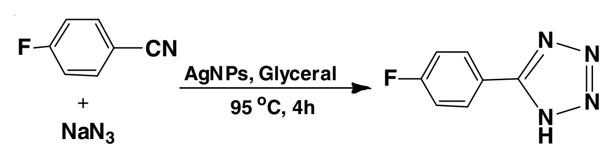
Vitex negundo root bark extract was used as reducing and stabilizing agent to synthesize silver nanoparticles. Analytical grade $AgNO_3$ was purchased from Sigma-Aldrich, Nutrient and Muller-Hilton agar from Himedia and DMSO was procured from SD Fine-Chem. Limited, India. The glasswares used for this study were cleaned with HNO_3 and distilled water. Root bark of *Vitex negundo* was collected in the vicinity of Punganur town, Chittoor District, India.

Preparation of root bark extract: The healthy root bark from *Vitex negundo* plant was collected for the green synthesis of silver nanoparticles. It was washed thoroughly with running tap water first and then with double distilled water and later dried for a few days properly at room temperature. The dried root bark was crushed into tiny pieces and grounded into fine powder. Fine powder (5 g) was dissolved in 100 mL of Milli-Q water in an Erlenmeyer flask. The above mixture is constantly rotated with the help of magnetic stirrer with a hot plate at 80 ºC for about 0.5 h. After it was allowed to room temperature the mixture was filtered by using Whatman filter paper No.1 and the filtrate was kept in refrigerator at 4 ºC for doing further experiments.

Preparation of silver nanoparticles: The root bark extract (20 mL) from *Vitex negundo* was added to 80 mL of 1 mM $AgNO_3$ solution in 250 mL Erlenmeyer flask followed by stirring at room temperature for 0.5 h with the help of magnetic stirrer. The change of colour of the solution from brown to dark brown was observed and kept under incubation for 24 h after covering the Erlenmeyer flask with silver foil. Finally, silver nanoparticles were collected through centrifugation and drying at hot air oven.

Characterization of silver nanoparticles: The absorption maxima of green synthesized nanoparticles were studied by UV-visible spectrophotometer at wavelength range from 200-700nm. FT-IR spectra were recorded in the range of 4000-400 cm^{-1} and the average particle size was measured with the help of XRD instrument by using a powder diffractometer. Morphological characterization of the sample was measured using scanning and electron microscopy and energy dispersive spectroscopy (EDS) techniques.

Preparation of 5-(4-fluorophenyl)-1*H*-tetrazole: Briefly, 4-fluorobenzonitrile (2.0 mmol), sodium azide (2.5 mmol) and AgNPs (20 mol %) were taken into a round-bottomed flask and dissolved with 5 mL of glycerol. The reaction mixture was stirred for 4 h at 95 ºC and examined the growth of reaction by TLC. Then after completion reaction, pH of reaction mixture was adjusted with 10 mL of HCl (0.1 N) and then extracted with ethyl acetate (3 × 18 mL). The crude product of 5-(4-fluorophenyl)-1*H*-tetrazole (**Scheme-I**) was obtained after washing with water, drying with anhydrous Na_2SO_4 and concentrated under reduced pressure. Finally, this crude product was purified by column chromatography using 50 % ethyl acetate:*n*-hexane as an eluent and subsequently characterized by mass, elemental analysis and ^{1}H NMR techniques. Colour: white solid, yield: 81 %, m.p.: 75-77 ºC, ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20-8.26 (m, 2H, J = 7.6 Hz, Ar-H), 7.50-7.59 (m, 2H, J = 7.6 Hz, Ar-H), 3.31 (br s, 1H, NH, overlap with solvent) ppm. LC-MS (positive) m/z (%): 165 $[M+H]^+$ (100 %). Anal. calcd. (found) for $C_7H_5N_4F$: C, 51.22 (39.80); H, 3.07 (2.28); N, 34.13 (38.45).



Scheme-1: Synthesis of 5-(4-fluorophenyl)-1*H*-tetrazole

Antibacterial assay: The AgNPs synthesized from *Vitex negundo* root bark extract were tested for their antibacterial activity by agar well diffusion method [30] with small modifications [31]. The bacterial strains used for examination was against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*. Seeded agar medium was prepared using Muller-Hilton, sterilized and allowed to cooling to room temperature. To this 0.1 mL of diluted in oculum (10^7 cfu/mL) of test organism was added just before solidification and then it was poured into sterilized plates under sterile conditions. Then wells of 4 mm diameter were punctured with cork borer maintaining aseptic environment. These wells were filled with 10, 20, 30 and 40 µL of nanoparticle solution and solvent DMSO is used as control. The plates were incubated at 35 ºC for 18 h and then measured the zone of inhibition (in mm) against test organisms. The standard drug benzyl penicillin at 20 µg/ mL concentration was used as positive control.

RESULTS AND DISCUSSION

Characterization of silver nanoparticles: Generally, in all experiments related to plant mediated synthesis of metal nanoparticles visual observation plays an important role to judge its formation. The colour change from brown to dark brown is noticed after treating the aqueous solution of plant extract with silver nitrate. It has been identified that the yields of silver nanoparticles depending on some parameters like concentration of silver nitrate, concentration of plant extract, time, *etc*. Silver nanoparticles were synthesized from root bark extract of *Vitex negundo* and 1 mM of $AgNO_3$ were analyzed by using UV-visible spectroscopy. It has been observed that a single plasmon resonance band observed at 433-443 nm (Fig. 1) confirmed the formation of AgNPs and no such peak was identified for UV spectra of pure plant extract.

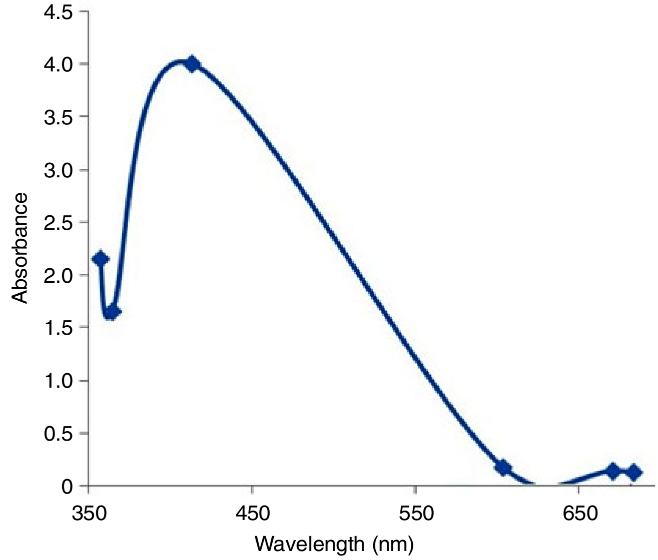


Fig. 1. UV-visible spectra of green synthesized AgNPs

FT-IR analysis for pure root bark extract and silver nanoparticles were carried out to confirm the presence of biomolecules and their dual role as reducing and capping agents. The spectrum of pure extract showed major absorption peaks at 3314 cm^{-1} (O-H *str*.), 2853 cm^{-1} (C-H *str*.), 1630 cm^{-1} (C=O *str*.), 1442 cm^{-1} (O-H *def*.) and 1074 cm^{-1} (Fig. 2a) confirms the presence of plant biomolecules. The absorption peaks present at 3358, 2862, 1650, 1470 and 1095 cm^{-1} (Fig. 2b) which revealed that biomolecules were bound to silver nanoparticles and act as capping agents. This results revealed that hydroxyl, carboxylic acid, amine or amide functional moieties were attributed to alkaloids, phenolic compounds, flavanoids and fatty acids of plant extract coated strongly on nanoparticle.

The crystalline nature of silver nanoparticles was confirmed by XRD studies and the pattern showed 2θ angles at the range of 38.41º, 44.53º, 64.72º and 77.96º corresponding to the 111, 200, 220 and 311 (Fig. 3) confirmed the reflection from face centered cubic stricture of silver crystal. From SEM images (Fig. 4), it can be noticed that a well defined triangular or spherical shaped AgNPs were formed without agglomeration. Finally the strong signals of silver element and all silver peaks were observed in the range between ~ 2.6-3.9 KeV on EDS spectrum of green synthesized silver nanoparticles (Fig. 4).

Catalytic activity: Silver nanoparticles were utilized as catalyst in (3+2) cycloaddition synthesis of 5-substituted 1*H*-tetrazole. The quest for use of green methods in synthesis we motivated to choose glycerol, a green solvent for the preparation of 5-(4-fluorophenyl)-1*H*-tetrazole in good yields. Herein, silver nanoparticles act as Lewis acid by activating nitrile group which enhances its electro philic character which later combines with sodium azide to produce tetrazoles [32]. The structure of newly synthesized tetrazole molecule was confirmed by ^{1}H NMR, mass spectral data and C,H,N analysis.

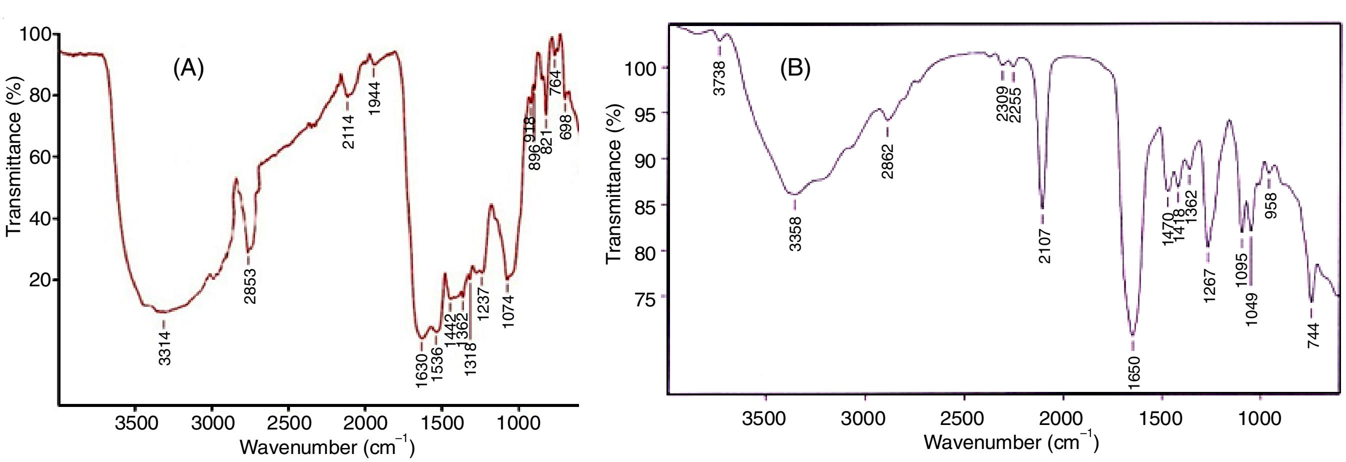


Fig. 2. FT-IR spectra of (A) Pure *Vitex negundo* root bark extract (B) AgNPs capped by *Vitex negundo* root bark extract

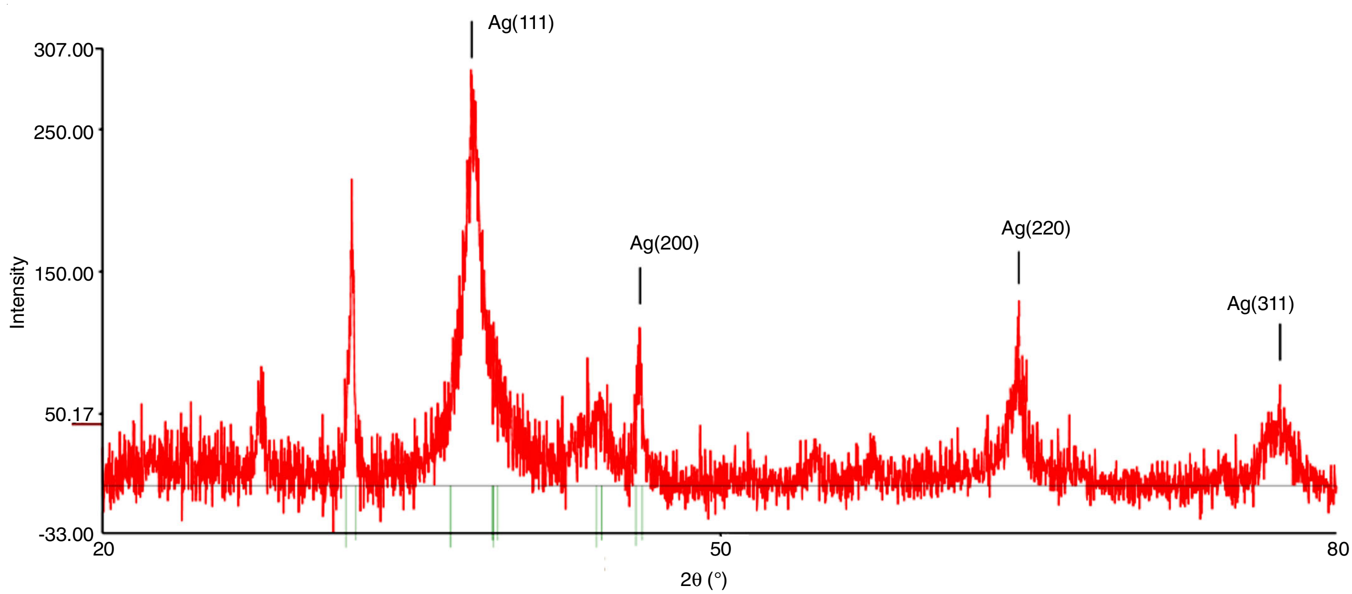
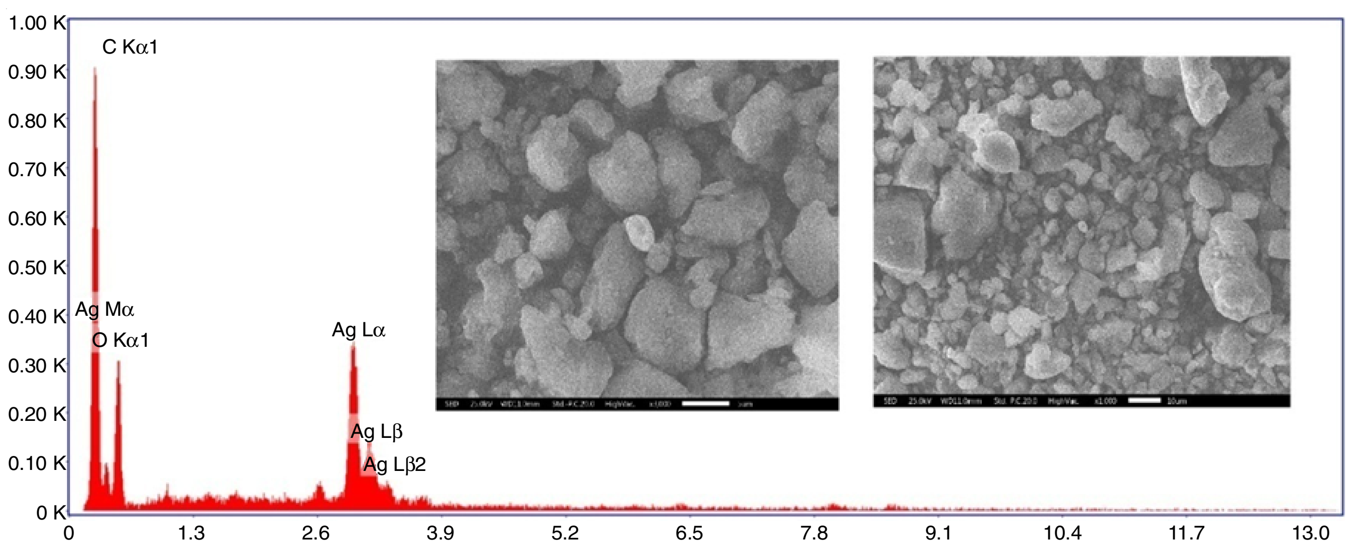


Fig. 3. XRD patterns of synthesized silver nanoparticles



eZAF Smart quant results

Element	Weight %	Atomic %	Net Int.	Error %	K ratio
C K	31.78	49.53	83.44	6.69	0.2260
O K	38.76	45.36	40.64	12.28	0.0645
Ag L	29.46	5.11	69.77	6.35	0.2500

Fig. 4. SEM-EDX analysis of synthesized silver nanoparticles

Antibacterial assay: The antibacterial assay of silver nanoparticles synthesized using *Vitex negundo* root bark extract was evaluated by agar well diffusion method. The biologically synthesized AgNPs have showed excellent range of activity at the concentration of 40 µg/mL against all the tested organisms such as *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*. The results of zone of inhibition (Table-1) was calculated and compared with standard drug benzyl penicillin. *Escherichia coli* (18 ± 1.6 mm) and *Bacillus subtilis* (16 ± 1.4 mm) showed excellent sensitivity (concentration of AgNPs: 40 µg/mL) when compared with *Staphylococcus*

TABLE-1
ANTIBACTERIAL ACTIVITY OF GREEN SYNTHESIZED SILVER NANOPARTICLES

AgNPs (µg/mL)	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*E. coli*
10	10 ± 1.1	10 ± 0.8	10 ± 1.2	11 ± 0.8
20	12 ± 0.2	12 ± 1.2	11 ± 1.1	13 ± 0.6
30	11± 0.6	14 ± 1.0	12 ± 0.5	14 ± 1.4
40	13 ± 0.5	16 ± 1.4	14 ± 1.2	18 ± 1.6
Std*	21 ± 1.5	23 ± 0.5	25 ± 1.0	22 ± 1.5

*Standard drug: benzyl penicillin

aureaus (13 ± 0.5 mm) and *Pseudomonas aeruginosa* (14 ± 1.2 mm). Further, it was explained that the antibacterial activity of silver nanoparticles is concentration dependant and works for both Gram-positive and Gram-negative bacteria.

Conclusion

The synthesis of silver nanoparticles by *Vitex negundo* root bark extract is reported *via* green chemistry approach which provides efficient, eco-friendly, cost effective ways for the synthesis of metallic nanomaterials. The characterization results confirmed that the reduction of silver ions to silver nanoparticles when it was treated with plant extract. This biogenic silver nanoparticles exhibited excellent catalytic activity for the formation of 5-substituted 1*H*-tetrazole in good yields. Finally, the antibacterial activity profile revealed their potentiality in biomedical applications.

ACKNOWLEDGEMENTS

The authors thank to Sri Jayachamarajendra College of Engineering, Mysore, India and Mr. C.M. Narendra Reddy, Department of Biotechnology, Dravidian University, Kuppam, India, for providing instrumentation facilities and antibacterial activities.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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