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In situ green synthesis of Cu-doped ZnO based polymers nanocomposite with studying antimicrobial, antioxidant and anti-inflammatory activities

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Abstract

The use of eco-friendly methods for the synthesis of nanoparticles and its nano-composite has become a public demand nowadays to reduce the risks of chemical methods. In the current study, green synthesis of Cu-doped ZnO based polymers nan-ocomposite was performed. Various instrumental analysis including UV–vis, ATR-FTIR spectroscopy, XRD, SEM coupled with energy dispersive X-ray analysis, TEM and Thermal gravimetric were used to characterize nano-composite. Highly antibacterial activity of the synthesized nano-composite was recorded against tested microorganisms with promising efficacy against bacteria namely; *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Proteus vulgaris, Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhimurium* and yeast (*Candida albicans*) but unfortunately not against black fungus (*Mucor circinelloides*) and filamentous fungi *Aspergillus flavus* and *A. niger.* Anti-inflammatory of nano-composite represented by hemolysis inhibition was observed at using low concentration (100 µg/mL) with enhancing 23.85% compared with free nano-composite while at high concentrations 500 and1000 µg/mL the anti-inflammatory activity was approximately similar with enhancing 3.91% and 1.99%, respectively. Antioxidant of the nano-composite was better than the antioxidant of free nano-composite at all tested concentrations, moreover the IC_{50} of the nano-composite (91.16 µg/mL) was less than the IC_{50}, (203.65 µg/mL) of the free nano-composite.

Keywords: Green synthesis, Cu-doped ZnO, Nano-composite, Antimicrobial activity

Introduction

Nanomaterial-based industries have received substantial attention from the scientific public for several reasons. The presence of materials in the nano-form made them to acquire a new properties and unique phenomena that were not present in its non-nano-scale [1]. These unique properties attracted the attention of scientists to how these materials can be exploited in many industrial and medical applications [2, 3]. Furthermore, the nano-composites of natural sources have more safety profile and it can support in biocompatibility improving. The natural polymers such as cellulose and gelatin as well as derivatives represents attractive compounds and play an important role in several biological applications. Cellulose was used as a dialysis membrane, encapsulating mediator for drug delivery, wound dressings and tissue engineering supports. Many applications of the carboxymethylcellulose (CMC) as a derivative of cellulose were reported like as coating fluids, cosmetics, drug supply, binders, textiles industry, paper and food biotechnology for several reasons such as little cytotoxicity, great water solubility, non-allergenicity, few immunogenicity, biodegradability and biocompatibility [4, 5]. Furthermore,

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composite matters based on natural polymers such CMC were suggested for some pharmaceutical applications. The build composites may enhancing the biocompatibility and mechanical properties of CMC [6].

Creation of nanoparticles (NPs) via green methods compared with chemical and biological methods becomes more prevalent and the main target of recent studies due to its incomparable benefits. Green methods are simpler, eco-friendly and cheaper, and do not leave any waste that disrupt the environment. From the green methods, ultrasonic-assisted, microwave assisted hydrothermal, sol-gel, and co-precipitation, microwave assisted hydrothermal, chemical vapor deposition, spray pyrolysis methods [7, 8]. Synthesis of NPs via mechanochemical methods of hybrid composites was also applied to reduce the hazards of pure chemical methods [9]. Ultrasonic was used as a green method of creation NPs doped with other compounds for enhancing degradation process of dyes and engorgement the antimicrobial activities [8, 10]. Sonochemical and microwave synthesis represent one of the common techniques in green synthesis of nanomaterials. Recently, investigators applied ultrasonic or microwave-assisted approach along with the usage of the natural polymers for the production of nano-materials. Ultrasound is the subset of the sonic spectrum that is universally used in Sonochemistry [7]. Microwaves are a subcategory of the electromagnetic spectrum (300 MHz up to 300 GHz) [7, 10]. Therefore, synthesis of nanocomposite in the current study was performed using Sonochemical and microwave methods.

In the current decade, NPs of CuO and ZnO is considered the main prevalent among metals due to their unique physical, chemical, and mechanical features, for example, structural stability, a larger surface area, low melting temperature, high diffusion, and high surface energy. ZnONPs characterized by electrical and optical properties make them appropriate for novel application and devices [11]. Photocatalysis, antibacterial and biochemical sensors were reported as promising applications of ZnONPs [12–14]. The low toxicity of ZnONPs attracted scientists to focus on its application in the pharmacy and biomedicine fields such as antibacterial and antifungal activities [15, 16]. Previously, ZnONPs were added to textiles for suppressing bacterial growth [17]. Previous texts described that ZnONPs inhibit bacterial growth by disrupting the membrane structure [18]. Sirel et al. [19] mentioned that particle size, crystallinity index, and optical characteristics of ZnONPs can be modified through doping with metal or non-metal. Enhancement of the activity of ZnONPs was reported in recent studies via combination with other metals [20, 21], therefore doping of ZnONPs with Cu as a transition metal has showed a significant effect on the physical properties. Antibacterial activity of ZnONPs against *Escherichia coli*, and *Staphylococcus aureus* were enhanced via doped with diverse metal ions [22, 23].

The combination of lignin with NPs of compound containing copper leads have stronger activity for the control of undesirable microorganisms [9]. Cu-doped ZnONPs are currently created by green methods. Advanced applications of ZnONPs were developed via doping with Cu [24]. Moreover, a more ionization energy and little formation energy were associated to Cu, leading to speeding the incorporation of Cu into the ZnO matrix. Green synthesis of Cu-doped ZnONPs was recorded [25], and its anticancer, antioxidant, antibacterial, antifungal, and photocatalytic activities were observed. Doping ZnONPs with Cu enhanced the activity, chemical and physical characteristics of ZnO [21, 26, 27]. Therefore Cu-doped ZnONPs are utilized in numerous fields including treatment of water from microbial pathogens contamination [28, 29]. The current study aimed to green synthesis of Cu-doped ZnO based polymers nano-composite and its medicinal application as antimicrobial, antioxidant and anti-inflammatory activity.

Materials and methods

Materials

Carboxymethylcellulose (CMC) was purchased from Sigma Aldrich Co. Gelatin type-B of bovine skin and Zinc acetate 99.99% trace metals basis were obtained from Sigma, Germany. Copper acetate monohydrate was purchased from Loba Chem, India. Other chemicals, culture media, and reagents used in this study were purchased from Modern Lab Co., India in analytical grade without any purification required.

Methodology

Synthesis of nano-composite

In this work, the novel method was used to synthesis the green nano-composite based polymers and doped by Cu-doped ZnO nanoparticles. Firstly, the polymers (CMC and Gelatin type-B) were dissolved with 1% (w/v) as well as the metal salts dissolved as 1 Mmol individually using Millipore water. The polymers solicitations were mixed 1:1 ratio and steering vigorously at 1500 rpm and 70 °C for 3 h. Then the collected solution was divided into two equal volumes which were processed with the same processes while one of them has not been containing metals and namely, free one (Free nano-composite). The other one process with adding metals salts were added by ratio 1:10 Cu to Zn solutions drop wise under the above conditions for overnight. The collected solution were sonicated for 15 min with 1000 W prop ultrasonic. The tannic acid were added as 2% of the whole final solution volume. The final product was microwaved for 3 min using 1000 W

microwave. The nano-composite was maintained in the refrigerator for further analysis and uses.

Synthesis of Cu-doped ZnO based polymers nano-composite characterizations

Investigation of the structural changes of different samples was performed by UV- visible spectrometer of type V 630 (Jasco, Japan). Fourier transform infrared spectroscopy-attenuated total reflectance (FTIR–ATR) spectroscopy (Spectrum Two IR Spectrometer—PerkinElmer, Inc., Shelton, USA). All spectra were obtained by 32 scans and 4 cm^{-1} resolution in wavenumbers ranging from 4000 to 400 cm^{-1}. The crystal structure was determined using X-ray diffraction analysis (XRD) (Model diffractometer, Shimadzu 7000, Japan.). The surfaces of prepared samples were investigated by a field emission Scanning electron microscope (SEM) coupled with energy dispersive X-ray analysis; Model Quanta 250 FEG (Field Emission Gun) attached with Energy dispersive X-ray spectroscopy (EDX) Unit (Energy Dispersive X-ray Analyses) for EDX and mapping, with accelerating voltage 30 kV. Transmission electron microscopy (TEM) of type JEM2010, Japan, was used to investigate particle size and morphology of the synthesized samples. The thermal gravimetric analysis (TGA) was also performed using a thermal analyzer (SDT Q600, USA).

Antioxidant activity of Cu-doped ZnO based polymers nano-composite

The Cu-doped ZnO nano-composite and free nano-composite were subjected to assessed its antioxidant activity via 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test protocol. Different dilutions of the synthesized nano-composite were prepared, 2 mL of the prepared DPPH (0.1 mM) in methanol was mixed with each dilution separately, followed by shaking with vortex, then preserved in a shade for 60 min. The reaction mixture of the negative control was performed via the addition of the prepared DPPH solution (2 mL) to 1 mL of methanol [30]. The reaction mixture was subjected to UV–visible spectrophotometer (Milton Roy, Spectronic 1201). The decline in absorbance (A) at 515 nm was recorded continuously at one min intervals pending the A is stabilized (16 min) to calculate radical scavenging (RS) (%) of DPPH via the next equation:

$$\text{RS of DPPH activity (\%)} = \frac{\text{A at control} - \text{A at treatment}}{\text{RA at control}} \times 100$$

Inhibitory concentration 50% (IC_{50}) of the treatment was recorded.

Antimicrobial activity of Cu-doped ZnO based polymers nano-composite

Disc diffusion method was used to test the killing effect of Cu-doped ZnO based polymers nano-composite against different microorganisms including filamentous black fungus (*Mucor circinelloides*), unicellular fungus (*Candida albicans*), mycotoxigenic fungi (*Aspergillus flavus* and *A. niger*) Gr + ve bacteria namely *Bacillus subtilis*

(ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 10541); and Gr−ve bacteria namely *Proteus vulgaris, Pseudomonas aeruginosa*, *Escherichia coli* (ATCC 8739), (*Salmonella typhimurium*). Disc (6 mm) was loaded with (100 µL, of 20 µg) of the tested compound, then potted on the streaking agar media with the tested organisms. For appropriate diffusion of the tested compounds, the plates containing tested microbes were reserved in the refrigerator for 25 min [3]. The activity of the tested compound was compared with the activity of antibiotic (Gentamycin) and antifungal (Ketoconazole). After the incubation period, the appeared clear zone around the discs was measured [31].

Anti-inflammatory via hypotonicity induced hemolysis of Cu-doped ZnO based polymers nano-composite

A blood sample (3 mL) was drawn from the authors of the current paper as healthy volunteers, then collected in heparinised tubes. At 3000 rpm, the blood sample was centrifuged for 10 min, then the collected supernatant was mixed with normal saline (v/v) to dissolve the pellets of blood. Sodium phosphate buffer (10 mM, pH 7.4) as isotonic buffer solution was added to the dissolved pellets (as 2:3 v/v). The re-suspended supernatant was used as erythrocyte suspension. Cu-doped ZnO based polymers nano-composite as a tested compound was suspended in distilled water for making hypotonic solution. Gradually concentrations of the hypotonic solution (HS) contains 100, 200, 400, 600, 800 and 1000 µg/mL were mixed separately (5 mL) with 0.1 ml of the erythrocyte suspension in centrifuge tubes, followed by mixing gently and then incubating at 37 °C for 60 min. At the same time, 5 ml of the isotonic solution (IS) containing the same concentration of the tested compound was potted in centrifuge tubes and treated as in HS. At the end of the incubation time, the mixture was centrifuged at 1300*g* for 3 min [32]. Via Spectronic (Milton Roy) spectrophotometer, the absorbance (OD at 540 nm) of the collected supernatant containing haemoglobin was read to estimate the

hemolysis % regarding 100% hemolysis using distilled water. The following formula was applied to detect % of hemolysis inhibition of (HI):

$$\mathrm{HI}(\%) = 1 - \frac{\text{OD of the test compound in HS} - \text{OD of the test compound in IS}}{\text{OD of the control in HS} - \text{ODof the test compund in IS}} \times 100$$

Statistical analysis

The ± standard deviation (SD) and ± standard Error (SE) means of three replicates were recorded. GraphPad Prism® (version 5.0) software was applied to calculate IC_{50} of the activity of DPPH radical scavenging.

Results and discussion

Characterizations of Cu-doped ZnO based polymers nano-composite were confirmed via numerous different techniques to characterize crystal structure, size, surface topography, elemental composition as well as other physical properties as follow.

UV–visible spectroscopic

The optical properties of the free and loaded nano-composites were investigated as shown in Fig. 1. It well known that most polymers do have not any optical properties in the visible as well as UV range spectrum [33, 34]. Moreover, the UV spectrum of the free nano-composite was performed with no detected peaks which refract to the nature of the native materials of the nano-composite. On the other hand, with the addition of the ZnO doped Cu the loaded nano-composite spectrum was observed a large absorption in the visible light region with broad peaks at about 300 and 400 nm which are due to the presence of ZnO doped Cu nanoparticle as mentioned previously [35].

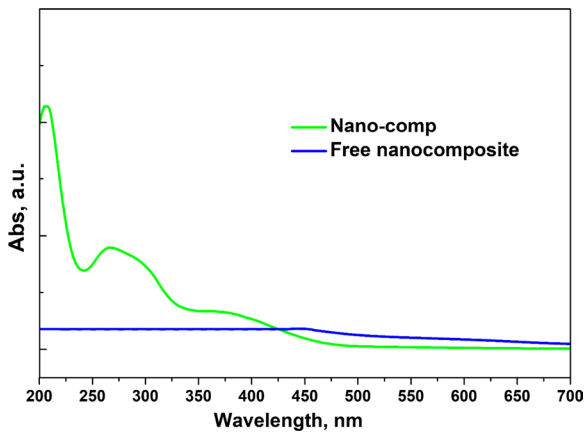

Fig. 1 The UV–vis spectra of Free and loaded nanocomposite

FTIR

FTIR is a tool used to study the intermolecular and intramolecular structure changes during the formation of materials. In this context, the FTIR spectra of native materials as well as synthesized nano-composites are presented in Fig. 2. The spectrum of native CMC has observed the characteristic bands at 3450, 2925, 2857 1621, 1425, 1330 and 1045 cm^{-1} were corresponding to starching vibration of OH, stretching vibration of CH, asymmetric and symmetric C–H stretching vibration, carboxyl group (–COO) [36, 37], scissoring of $–CH_2$, scissoring of –OH and glycosidic linkage of polysaccharide, respectively [38]. Additionally, the behavior of the FTIR spectrum of native gelatin consists of the three major regions reflecting the amide A and B; also, amide I to amide VI which is observed clearly in this spectrum [39]. Herein, the bands at 3313, 2940, 1712, 1424, 1331, 1250 and 1035 cm^{-1} were attributed to NH bond in the peptide group and for the OH of carboxylic group, $–CH_3$ & $–CH_2$ stretching vibrations, C=O overlapping between carbonyl groups, CH_2, $–CH_3$ bending vibration and C–O–C of the amide bond stretching vibrations, respectively [40]. Whereas, the formation of nano-composite loaded ZnO doped Cu nanoparticle is modified the FTIR spectrum in comparison with the native nano-composite components. Moreover, the many characteristics bands were observed with significant changes in the loaded nano-composite spectrum. At the same time, the

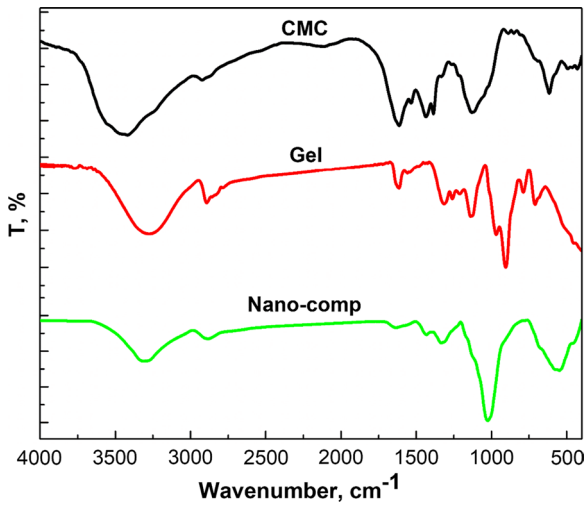

Fig. 2 FTIR spectra of native materials as well as synthesized nanocomposites

loaded nano-composite hydroxyl groups were assigned at 3321 cm^{-1} with new position not assigned in the nano-composite native materials spectra. In addition, C–H stretching vibrations band was observed at 2892 cm^{-1} with the high shift to a low frequency which is due to involved of these group in green reducing as well as capping nanoparticles. In this context, the band of CMC carbonyl group was shifted to high frequency as results to in situ formation of nanoparticles. Otherwise, the band of the nanometals was observed at 448 cm^{-1} as a new band with confirmation of the ability of the native polymers to reduce the metals to nanosized [41]. Overall, these observations were after mind that the native polymers are considered as reducing/capping agents with good ability, and different performance physicochemical properties which can be summarized as green and ecofriendly effective nanotechnology method.

XRD

The crystallography of native polymers materials, as well as loaded nano-composite were tabulated in Fig. 3. The pure CMC crystallography pattern was illustrated as a typical polymers with single broad band at about 20^{O} [42]. In addition, the pure gelatin pattern indicates the amorphous structure like most natural biopolymers with the single heap at around $_{17}O$ [43]. On the other hand, the loaded nano-composite was observed as amorphous in the organic region at about 19. Otherwise the peaks at 37, 42, 44, 51, 55 and $_{66}O$ were attributed to the ZnO doped Cu as a crystal particle with low concentration which shows high similarity with peaks indexed of card JCPDS no. 01-089-1397 which corresponds to the formation of the hexagonal wurtzite structure with small linear variation in lattice parameters as result to present of cupper ion with low concentration doping [44].

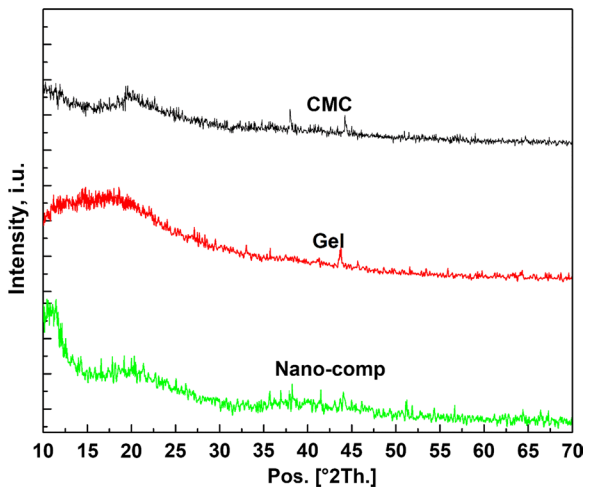

Fig. 3 Crystallography pattern of native materials as well as synthesized nanocomposites

SEM

The topographical study was carried out using SEM for free and loaded nano-composite. Figures 4A, B illustrated the morphology of the surface of the nano-composite after and before loading of ZnO doped Cu nanoparticles. The free nano-composite (Fig. 4A) observed a surface morphology typical polymer appearance with unique aggregations which is referred to as nanostructure. Whereas, the addition of ZnO doped Cu nanoparticles significantly effects the surface morphology where the surface of loaded nano-composite was observed as rough and the metallic chain aggregations were observed clearly. Otherwise, the EDX chart (Fig. 4 C) of loaded nano-composite was assigned the presence of C, O and N atoms due to the polymers composition. Additionally, the mapping images (Fig. 4D, E) confirmed a good distributions of both metals ions with an increase in the Zn ions ratio. These results are affirmed that the nano-composite at the free state is performed as a nanostructure surface appearance with aggregations. On contrary, the loaded nano-composite is performed as a nanostructure surface with low aggregations in comparison with the free one due to the action of the ZnO doped Cu nanoparticles. Moreover, the EDX and mapping charts confirmed the presence of the metals involved in the polymers composition with the ratios added in the synthesis process approximately.

TEM

The TEM images for loaded nano-composite as well as the particle size distribution were observed in Fig. 5. The low magnification TEM image in Fig. 5A illustrated the morphology of the particle as nanostructure with particle clearly appears. Consequently, the moderate magnification image in Fig. 5B was clear the details structure of the nanoparticles as low aggregated crystals where there are arranged together as the nanoparticles nature when present in the polymers matrix which acts as the regulator dispersed phase. Moreover, the high magnification image was performed on the single particles with the dual shadow appearance where the particles were clear as plate doped with different dark grade spots that referred to the nanometal and doped. Afterward, the particle size distribution in Fig. 5C affirmed the nanostructure of the loaded nano-composite with a particle size around 60 nm. In fact, the nanosize of the ZnO doped Cu NPs was predicted to be small then the detected size via TEM while the nano-polymers are usually detected in nanosize around 100 nm. These observations are clear that

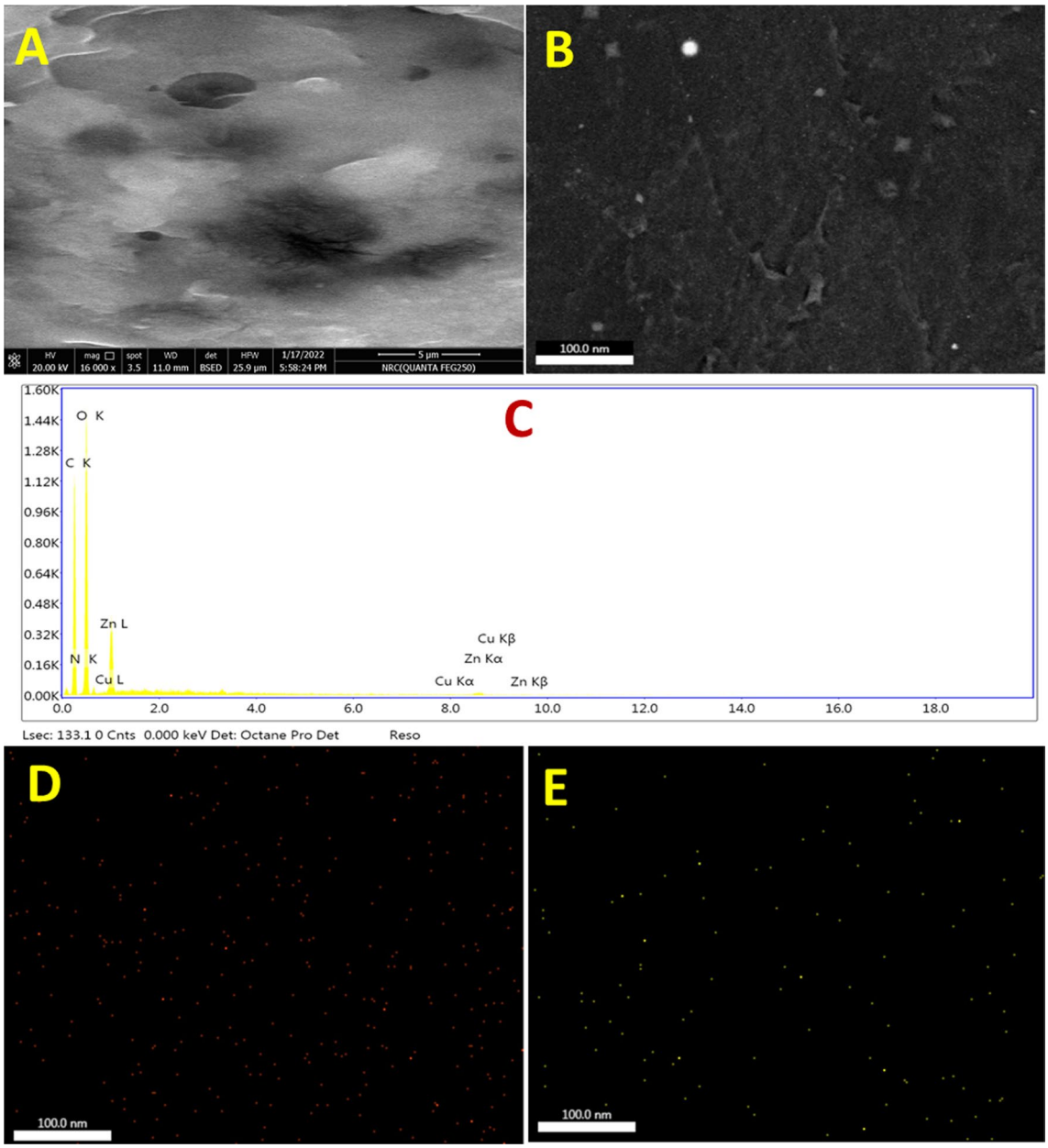

Fig. 4 Topography study of the free nanocomposite **A**, loaded **B**, EDX **C**, mapping of Zn ion **D** and Cu ion **E**

the nano-composite was prepared via green methods and doped with the nanostructure of ZnO doped Cu.

Thermal analysis

Figure 6 illustrates the thermal degradation of the free and loaded nano-composites. The free nano-composite observed moderate thermal stability in comparison with the known behaviors of the polymers. Otherwise, the loaded nano-composite was performed the excellent thermal stability in comparison with the free one. Moreover, the degradation stages of both appear typical in the first region before 300 °C. Whereas, after 350 °C the free nano-composite was decompose faster with the end weight remaining less than 10% at 800 °C due to the ash content of the raw materials. On the other hand, the loaded nano-composite performed good stability at elevated temperatures with a high remaining

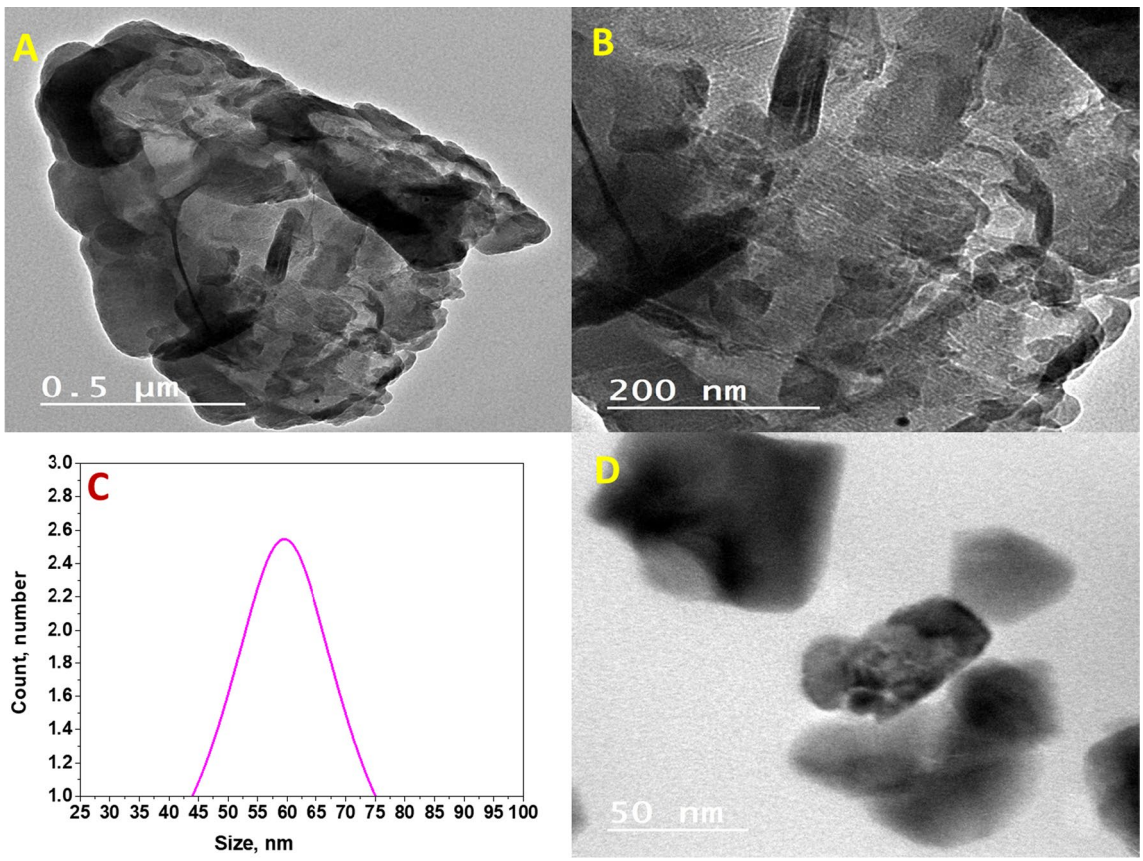

Fig. 5 TEM images via low magnification (**A**), high magnifications (**B**, **D**) and particle size distribution (**C**)

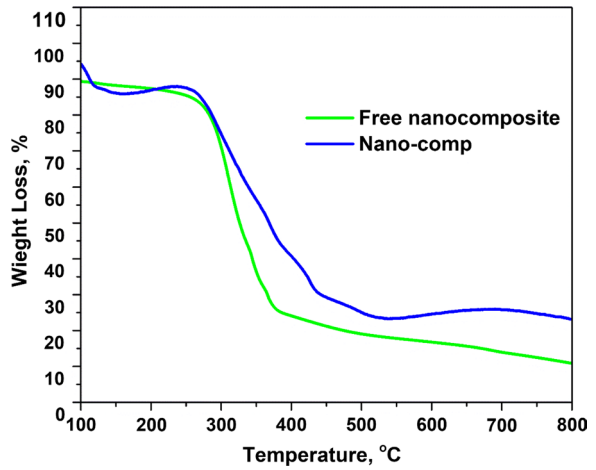

Fig. 6 Thermal gravimetric analysis of free and loaded nanocomposites

weight which reached more than 35% at 800 °C due to the nanostructure of involved metals.

Antimicrobial activity

Minimization of metals in the biological processes has become a fundamental topic for numerous reasons including negative health as well as environmental impact [9], hybrid among organic and inorganic materials become a critical issue for metals minimization. The comparative study of the efficacy of Free nano-composite and nano-composite against the growth of different microorganisms was reported in Table 1 and Figs. 7, 8.

The main inhibition zone was 27.17 ± 0.29, 44.38 ± 0.29, 24.93 ± 0.12, 27.90 ± 0.17, 27.93 ± 0.12, 25.07 ± 0.12, 34.93 ± 0.12 and 35.13 ± 0.23 mm using nano-composite while it was 20.93 ± 0.12, 30.07 ± 0.12, 19.27 ± 0.64, 20.97 ± 0.06, 19.07 ± 0.12, 22.5 ± 0.50, 31.0 ± 0.20 and 20.93 ± 0.12 mm using positive control

Table 1 Antimicrobial activities of free nano-composite and Cu-doped ZnO nano-composite

Test organism	Antimicrobial activity (mm)		
	Free nano-composite	Nano-composite	Control
Bacillus subtilis (ATCC 6633)	17.93 ± 0.12	27.17 ± 0.29	20.93 ± 0.12
Staphylococcus aureus (ATCC 6538)	20.33 ± 0.58	25.07 ± 0.12	22.5 ± 0.50
Enterococcus faecalis (ATCC 10541)	18.90 ± 0.17	24.93 ± 0.12	19.27 ± 0.64
Proteus vulgaris	18.07 ± 0.12	27.90 ± 0.17	20.97 ± 0.06
Pseudomonas aeruginosa (ATCC 90274)	20.0 ± 0.50	27.93 ± 0.12	19.07 ± 0.12
Escherichia coli (ATCC 8739)	32.63 ± 0.71	44.38 ± 0.29	30.07 ± 0.12
Salmonella typhimurium	27.07 ± 0.12	34.93 ± 0.12	31.0 ± 0.20
Candida albicans (ATCC 10221)	24.17 ± 0.29	35.13 ± 0.23	20.93 ± 0.12
Mucor circinelloide	0.0 ± 0.00	0.0 ± 0.00	20.87 ± 0.23
Aspergillus niger	0.0 ± 0.00	0.0 ± 0.00	18.83 ± 0.29
Aspergillus flavus	0.0 ± 0.00	0.0 ± 0.00	22.83 ± 0.29

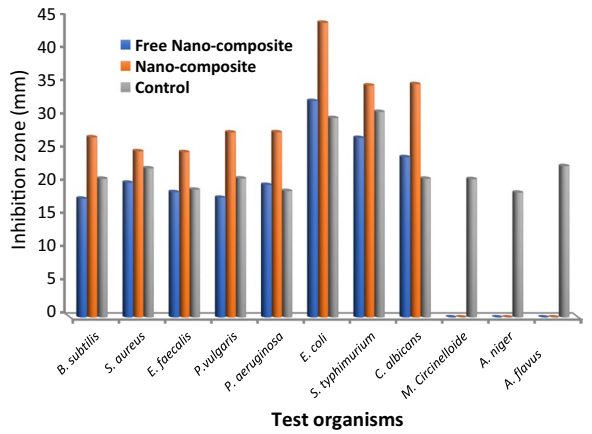

Fig.7 Antimicrobial activities of free nano-composite and Cu-doped ZnO nano-composite

(gentamicin against bacteria and fluconazole against fungi) against *B. subtilis*, *E. coli*, *E. faecalis*, *P. vulgaris*, *P. aeruginosa*, *S.aureus*, *S. typhimurium* and *C. albicans*, respectively. Surprisingly the antimicrobial efficacy of nano-composite was best than the positive control with enhancing the inhibitory activity that reached to 22.97, 32.24, 22.70, 24.83, 31.72, 10.25, 11.25 and 40.42% against *B. subtilis*, *E. coli*, *E. faecalis*, *P. vulgaris*, *P. aeruginosa*, *S.aureus*, *S. typhimurium* and *C. albicans* respectively. Nano-composite showed promising antimicrobial efficacy against tested bacteria and *C. albicans* but unfortunately didn't exhibit any inhibition activity against filamentous fungi (Table1 and Figs.7, 8), at the same time free nano-composite exhibited antimicrobial activity but less than nano-composite.

In a recent study, the appeared inhibition zone as a result of exposure *E. coli*, *K. pneumoniae*, *S. aureus* and *S. pyogenes* to nano-composite was 18, 11, 24 and 19 mm, respectively [45]. In the same context, Khalid et al. [45] demonstrate that nano-composite exhibited the highest antimicrobial activity than free nano-composite. From the current result, Gr + ve bacteria (*B. subtilis*, S. aureus and *E. faecalis*) were less sensitive than Gr −ve bacteria (*P. vulgaris*, *P. aeruginosa* and *S. typhimurium*) to free nano-composite unlike the obtained results of Khalid et al. [45]. Data in Table 1 showed that *C. albicans* next *B. subtilis* was highly susceptible to nano-composite. In a recent study, Hemaid et al. [46] reported the sensitivity of different species including *C. albicans*, *C. parapsilosis, C. glabrata* and *C. krusei* to 100 ppm of ZnONPs. The susceptibility or resistance may be due to dissimilarities between the walls structure of Gr −ve and Gr + ve bacteria. Hassan et al. [47] stated that the efficacy of nano-composite on bacteria resulted from releasing of Zn^{2+} and Cu^{2+} ions from nano-composite that induce the creation of reactive oxygen species. The obtained data are documented by other reports demonstrating that nano-composite exhibit excellent antibacterial efficacy than using ZnO [45, 47, 48]. A comparative study was performed by Rishikesan et al. [49] among the antimicrobial efficacy of ZnO and Cu-doped ZnONPs, who showed better activity of the latest. As mentioned previously, doping using transition metals modified the surfaces of nano-composite to provide it good biocompatibility, modify the chemical and physical illustrates [50]. Composite of Polysaccharide (lignin) with nanostructure of fungicide containing copper giving great fungistatic activity [9].

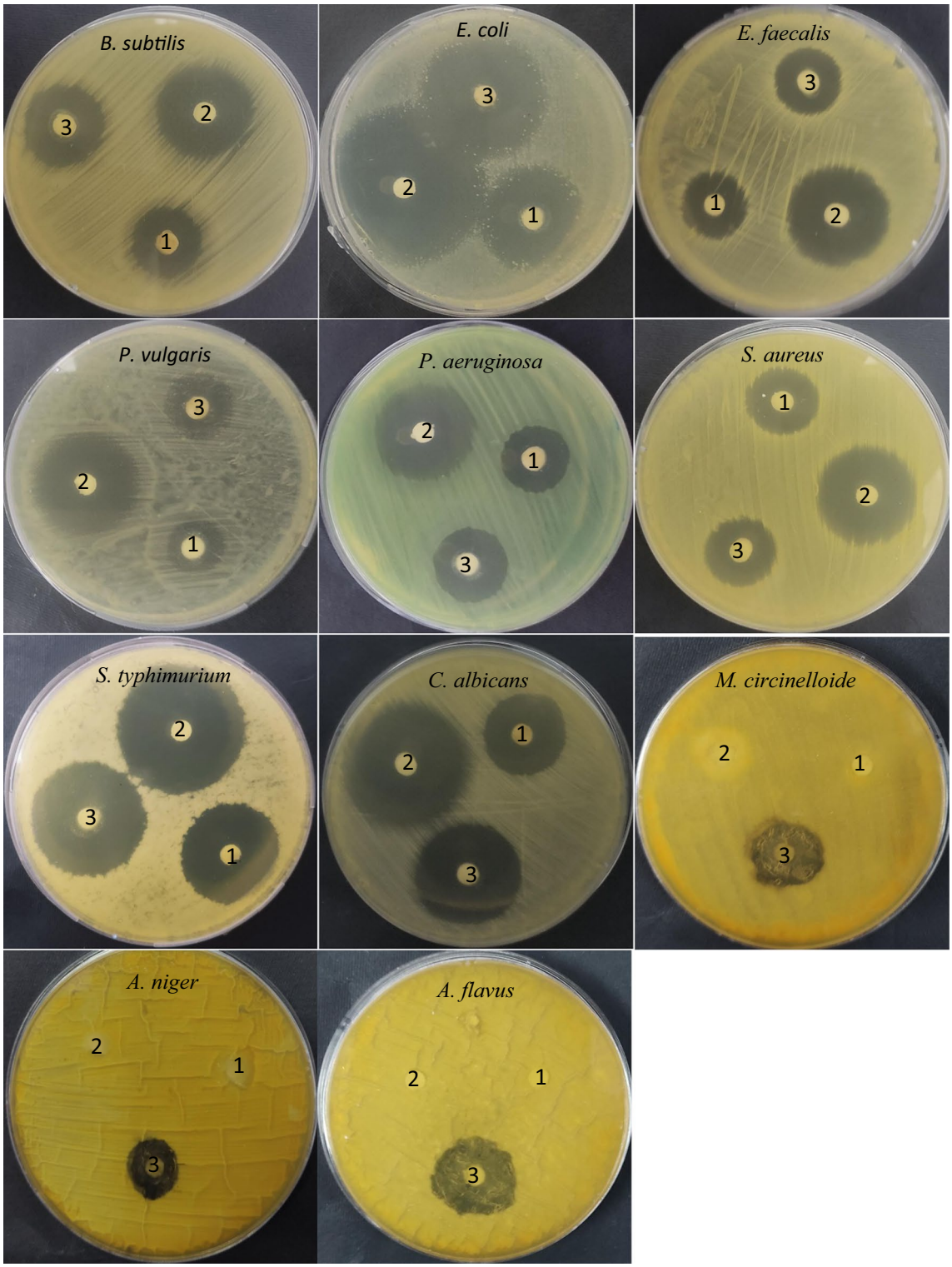

Fig.8 Antimicrobial activities of Free nan-ocomposite (1), Nanocomposite (2) and antibiotic (Gentamycin)/antifungal (Ketoconazole) (3)

Table 2 Anti-inflammatory activities of free nano-composite Cu-doped ZnO nano-composite

Concentration (µg/mL)	Free nano-composite			Nano-composite		
	HI %	SD	SE	HI %	SD	SE
1000	73.9	0.006	0.002	75.4	0.002	0.001
800	66.3	0.006	0.002	69.0	0.004	0.001
600	56.2	0.006	0.002	62.5	0.007	0.002
400	47.8	0.008	0.003	55.1	0.004	0.001
200	35.8	0.005	0.002	46.0	0.005	0.002
100	26.5	0.004	0.001	34.8	0.007	0.002

Anti-inflammatory

However the free nano-composite and nano-composite possess anti-inflammatory potential, but nano-composite enhanced the anti-inflammatory activity represented by hemolysis inhibition. Generally, the hemolysis inhibition % increased with increment concentration in a dose-dependent manner. The obtained finding are summarized in Table 2. The hemolysis inhibition % by 100 µg/mL of free nano-composite and nano-composite was 26.5% and 34.8% with enhancing 23.85%, respectively. At 200 µg/mL the hemolysis inhibition % was 35.8% and 46.0% with enhancing 22.17%, while the hemolysis inhibition % was 66.3% and 69.0% with enhancing 3.91%, at 800 µg/mL and was 73.9% and 75.4% with enhancing 1.99%, 1000 µg/mL of free nano-composite and nano-composite, respectively. The enhancing% of hemolysis inhibition was observed particularly at low concentrations. Alavi and Nokhodchi [51] mentioned that coupling of ZnONPs as nano-composite is another mode of enhancing biological activity such as antibacterial and anti-inflammatory. Moreover, the highest anti-inflammatory properties of ZnO nanoparticles was observed recently [52, 53].

Antioxidant activities

The antioxidant of free nano-composite and nano-composite increment with the increased concentration in a dose-dependent manner (Table 3), but the antioxidant of the nano-composite was better than antioxidant of free nano-composite at all used concentrations. For example at 250, 500 and 1000 µg/mL, the DPPH scavenging% was 51.8, 60.2 and 60.9% using free nano-composite, while it was 67.7, 77.8 and 79.9% using nano-composite. IC_{50} (91.16 µg/mL) of nano-composite was less than the IC_{50} (203.65 µg/mL) of free nano-composite indicating the efficacy of nano-composite. According to Rishikesan et al. [49] Cu-doped ZnONPs were subjected to the antioxidant activity via DPPH assay, the detected IC_{50} was 76.29 µg/mL, furthermore the observed lowest and highest % of inhibition against DPPH radicals was 12.54 and 59.45% at 20 µg/mL and 100 µg/mL, respectively.

Table 3 Antioxidant activities of free nano-composite Cu-doped ZnO nano-composite

Conc. (µg/mL)	Free nano-composite				Nano-composite			
	OD Mean	DPPH Scavenging %	SD	SE	OD Mean	DPPH Scavenging %	SD	SE
1000	0.434	60.9	0.005	0.002	0.376	79.9	0.011	0.004
500	0.573	60.2	0.001	0.000	0.492	77.8	0.004	0.001
250	0.695	51.8	0.003	0.001	0.609	67.7	0.006	0.002
125	0.817	43.3	0.008	0.002	0.763	47.1	0.001	0.000
62.5	0.939	34.8	0.009	0.003	0.863	40.1	0.004	0.001
31.25	1.027	28.7	0.005	0.001	0.942	34.6	0.004	0.001
15.63	1.115	22.6	0.008	0.003	1.021	29.1	0.005	0.002
7.81	1.238	14.1	0.003	0.001	1.138	21.0	0.004	0.001
3.9	1.360	5.6	0.005	0.001	1.262	12.4	0.013	0.004
1.95	1.428	0.9	0.003	0.001	1.349	6.4	0.002	0.001
IC_{50}	203.65 µg/mL				91.16 µg/mL			

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Author contributions
AMHAR conceptualization the study and writing—manuscript and editing, RY and MMB; performed study methods, RY writing the paper; ATM. writing—original draft preparation. All authors have read and agreed to the published version of the manuscript. All authors read and approved the final manuscript.

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Declarations

Competing interests
All authors declare no competing interests.

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