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Grafting of curcumin derivative on graphene nanosheets: investigation the antibacterial and antioxidant properties

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ABSTRACT

Curcumin and its derivatives were considered as the potent bioactive compounds. In the present study, a curcumin derivative was grafted on graphene surface to form the new hybrid GN-CP. Fourier-transform infrared spectroscopy and scanning electron microscopy confirmed the formation of synthesized nonomaterial. The antibacterial and antioxidant activities of nanocomposite were conducted in vitro assays, and results showed that GN-CP had better antibacterial activity and acceptable antioxidant capabilities in compare of curcumin and graphene.

Keywords: Graphene, Curcumin, Nanomaterial, Antibacterial, Antioxidant

Introduction

Graphene based materials are the two-dimensional monolayers of carbon, with the outstanding physicochemical properties including, large theoretical specific surface area, chemical stability, low cost, and wide availability [1]. Graphene based materials has attracted considerable attention over the last two decades for applications in many fields of medical such as biosensors, bioimaging, antibacterial and drug delivery [2, 3]. Typically, GO is synthesized by chemical exfoliation of graphite powders using strong oxidants such as $KMnO_4$ in concentrated H_2SO_4. The modified Hummers method is an example of these oxidizing processes [4]. During the process, the graphene sheets are torn into smaller pieces and obtained the oxygen-containing groups. In addition to having many wonderful features, it is possible to introduce some new properties by functionalization of graphene based materials. The surface of GO is highly adaptable because the presence of the oxygen functional groups which, allows the use of several functionalization approaches including covalent and noncovalent methods or decoration of nanoparticles [5-7]

The antibacterial activity is one of the interesting properties of graphene based materials. It has been showed that physicochemical properties of graphene such as size, morphology, dispersibility, and surface functionality, can affect its antibacterial activities [8]. Three cytotoxicity mechanisms for antimicrobial property of graphene based materials have been proposed. Mechanical destruction of the cellular membrane, derived from direct contact with graphene layer and action of sharp edges, is the first suggested mechanism. Oxidative damage, which can oxidize bacterial lipids, proteins, or DNA, is the second antibacterial mechanism. Wrapping the cellular membrane, which resulted from two dimensional nanosheet and inactivate or kill the bacteria, is other proposed mechanism [9].

Curcumin with the IUPAC name 1, 7-bis [4-hydroxy-3-methoxyphenyl]-1, 6-heptadiene-3, 5-dione, is a member of polyphenols with the remarkable biological activities. Curcumin and its metal complexes have attractive interesting for applying in many fields due to antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal and many more properties [10]. Even with all the excellent properties of curcumin, it has limitations such as low water solubility and stability under physiological conditions and rapid metabolism in body. Overcome these problems via modifying the structure, synthesizing the different derivatives or smart drug delivery systems has been the goal of many studies [11].

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Quaternary ammonium compounds (QAC) with general formula $R_4N^+X^-$ are excellent antimicrobial agents against bacteria especially Gram-positive organisms. QACs due to high antimicrobial activity and low toxicity have wide commercial applications in preparation of dyes, textile, sanitizing food, mouthwashes, toothpaste and breath sprays. Due to its positive charge, QAC is placed on the negatively charged surface of microorganisms through electrostatic force and interact with cell membranes, which result to disturbing bacterial lipid bilayer membranes and leakage of the cellular content [12]. According to this point that Gram-negative bacteria have a persistent layer so-called the outer membrane, QACs show less effect on these bacteria compared to Gram-positive bacteria.

In this study, the object is to obtain a compound that has an acceptable activity against both Gram-positive and Gram-negative bacteria. For this purpose, GO was functionalized by amin groups. On the other hand, a QAC derivative of curcumin with strong activity against *Staphylococcus aureus* (*S. aureus*) was synthesized, and then grafted to aminated GO by imine-bond formation. The resulting nanohybrids were studied for antibacterial properties against Gram-positive and Gram-negative bacteria. Since curcumin-related compounds have potent antioxidant properties, the nanohybrid was also investigated for this activity.

Experimental

Materials and instruments

Curcumin, 4-pyridinecarboxaldehyde, 1-bromodecane, Graphite, $KMnO_4$, ethylenediamine (EDA), Mueller-Hinton agar, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), and solvents were obtained from the Merck.

FT-IR spectra were recorded using a Shimadzu 8400 S spectrometer at the range of 4000 to 400 cm^{-1}. The samples were mixed with KBr powder at the ratio of 1:40. Ultraviolet–visible (UV–vis) analysis was performed on the Shimadzu 1650 spectrophotometer. ^1H Nuclear magnetic resonance (^1H NMR) spectrum was obtained in deuterated dimethyl sulfoxide (DMSO-*d*6) as the solvent using a Bruker (DRX-400 Avance) NMR spectrometer. An ultrasonic bath (Model: 5RS, 22 KHZ) was used to disperse the materials in solvents. Dried samples were coated with a thin layer of gold by sputtering for 15 s, and morphology investigation was performed using a LEO 440i scanning electron microscope (SEM) under vacuum at an operating voltage of 10 kV.

Synthesis of GN

First, GO was prepared from graphite according to an improved Hummers' method [4]. Then the aminated-GO (GN) was prepared from GO. For this purpose, 0.5 g GO was spread in 2-butanol (50 mL) and a uniform brown colloidal solution was obtained by sonication for 60 min. The solution was added slowly to excess amount of EDA (10 mL). The mixture was heated and stirred for 8 h in 80-85 °C, then cooled down to room temperature, and centrifuged (6000 rpm for 10 min). The residual solid material was washed with ethanol and centrifuged again (two times). The resultant solid of GN was dried at room temperature.

Synthesis of CP

First based on our previous method, 4-pyridinecarboxaldehyde alkylated by 1-bromodecane, and formed the 4-formyl-1-decyl pyridinium compound [13]. Then, a solution of pyridinium compound (0.446 g) was dissolved in 20 mL methanol (MeOH) and added to curcumin solution (0.5 g in 20 mL MeOH). 0.25 mL pyridine was added to the solution as the catalyst, and stirred at 65 ºC for 48 h. The dark red solution was cooled to room temperature, and the resulting red-brown product was washed with *n*-hexane, ethyl acetate, and diethyl ether to obtain CP

Synthesis of GN-CP

200 mg GN was dispersed in 50 mL *N*, *N*′-dimethylformamide (DMF) and a homogeneous solution was obtained by sonication for 30. Then, 200 mg CP in 10 mL DMF/MeOH and 0.5 mL formic acid was added to GN solution. The mixture was stirred at 70 °C for 48 h. The resultant GN-CP was centrifuged, and the supernatant was washed with MeOH a few times and finally, dried at room temperature.

Antibacterial and antioxidant activities assays

The Gram-positive bacteria *Staphylococcus aureus* (*S. aureus*) (PTCC 1112) and Gram-negative bacteria *Escherichia coli* (*E. coli*) (PTCC 1330) were chosen for cell viability test. The bacteria cells (10^6 to 10^7 CFU/mL) in isotonic saline were prepared and incubated with dispersions of compounds (40 μg/mL) solutions for 2 h under 250 rpm shaking speed. Antibacterial activities were investigated by the colony counting method described in the literature [14]. The isotonic saline solution without any material was used as the control. Antioxidant activity of the curcumin derivatives was determined using scavenging of DPPH free radicals [15].

Results and Discussion

Synthesis and characterization

New quaternary pyridinium derivative of curcumin named CP was synthesized and grafted on the surface of aminated graphen by imine linkage. The synthetic pathway is shown in Fig. 1.

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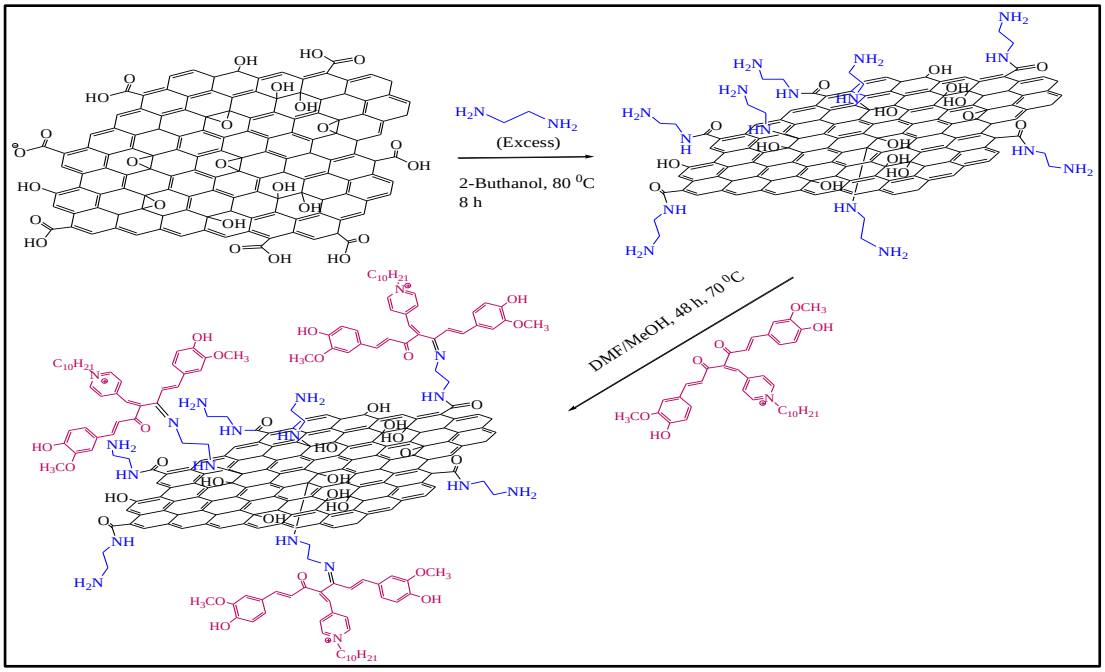


Fig. 1. Synthesis of GN-CP nanohybrid.

Characterization

After successful synthesis of compounds, they were characterized using various techniques including FT-IR, UV–vis, ^{1}H NMR, and FE-SEM spectroscopy. Fig. 2 shows the FT-IR spectra of GN, CP, and GN-CP compounds. FT-IR spectra of GN indicated N–H stretching bands as two weak bands at 3411 and 3431 cm^{-1} on the broad O–H band (3000–3600 cm^{-1}). In GN spectrum, the presence of two bands at 1635 cm^{-1}(C=O of the amide bond) and 1361 cm^{-1} (C–N) indicates the complete grafting of EDA on the GO surface. The spectrum of CP showed the absorption bands at 3400 cm^{-1} (O–H), 1731 cm^{-1} (carbonyl group), 1595 cm^{-1} (C–C conjugated diene), 1514 cm^{-1} (C–C aromatic) and 1153 cm^{-1} (C–O). Also, two sharp peaks at 2854 cm^{-1} and 2923 cm^{-1} are related to the *n*-decyl chain. Compared to GN, in the FT-IR spectrum of GN-CP, the presence of new absorptions at 2848 and 2920 cm^{-1} (aliphatic C–H) and 1734 cm^{-1} for (C=O) indicates the formation of GN-CP. This spectrum also showed the absorption bands at 3400 cm^{-1} (O–H), 1630 cm^{-1} (C=N along with C=O of amide), 1286 cm^{-1} (C–N), 1145 cm^{-1}, and 1107 cm^{-1}(C–O).

Fig. 3 shows the UV–vis spectra of GO, CP, and GN-CP compounds. Graphene oxide showed a peak at 243 nm and a shoulder at about 300 nm, which corresponded to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, respectively. CP showed two bands at 259 nm and 421 nm, which are the characteristic peaks of curcumin moiety. GN-CP nanomaterial demonstrated these specific absorption peaks with slight red shifts, confirming grafting of CP to surface of graphene nanosheet.

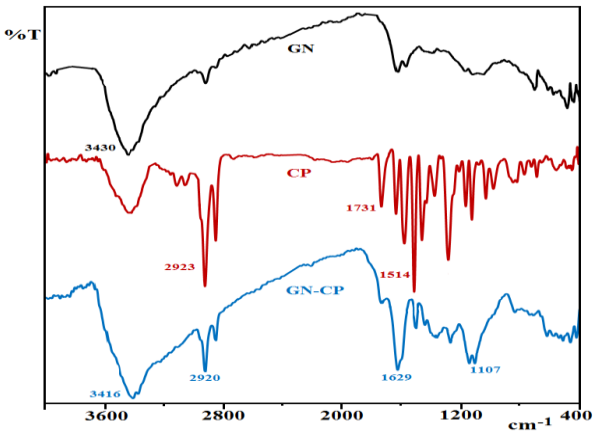


Fig. 2. FT-IR spectra of GN, CP and GN-CP.

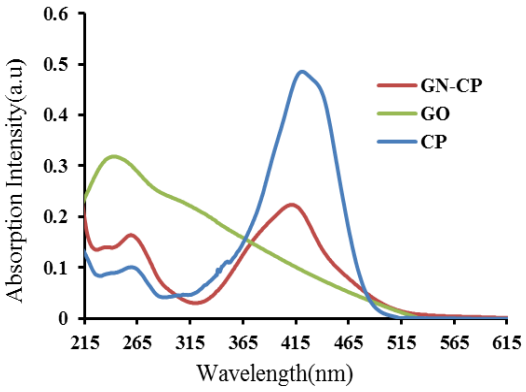


Fig. 3. UV-vis spectra of GO, CP and GN-CP.

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The structure of CP was characterized by ^{1}H NMR spectroscopy (Fig. 4). It showed the signals at δ = 7.56–6.78 ppm for the aromatic and double bond protons. The hydroxyl and methoxy protons of CP appeared at δ = 9.7 ppm and δ = 3.8 ppm, respectively. The doublet signals at δ = 9.28–9.25 ppm and δ = 8.49–8.46 ppm regions corresponded to the pyridine ring. The signals at δ = 4.67 ppm, 1.90 ppm, 1.22 ppm, and 0.83 ppm were attributed to the n-decyl chain bonding to the pyridine.

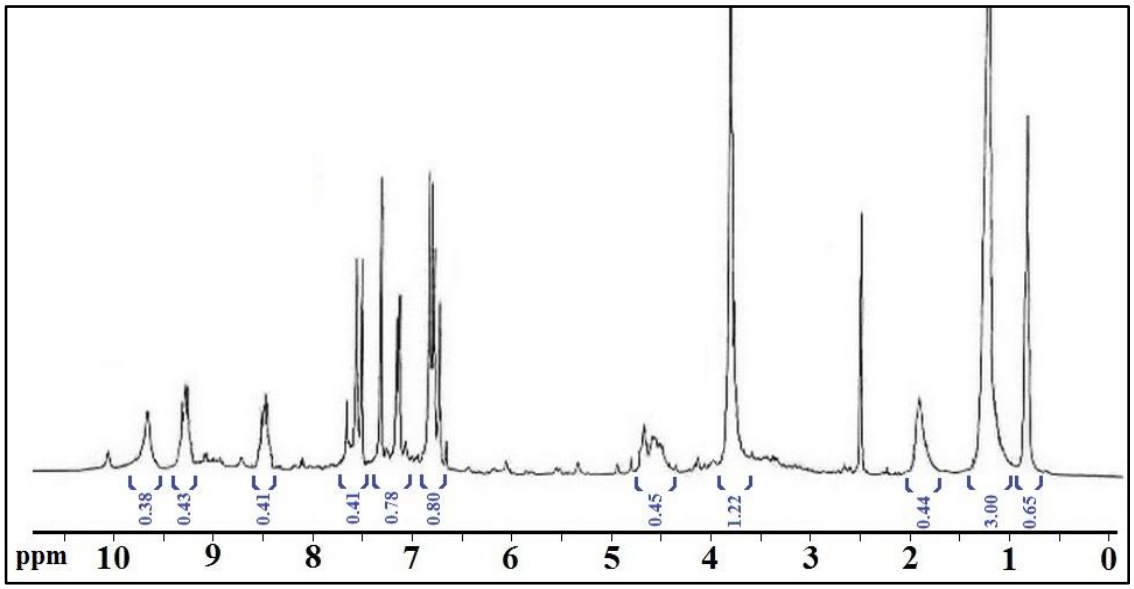


Fig. 4. ^{1}H NMR spectrum of CP in DMSO-d_6.

Morphology of nanomaterials was detected by field emission scanning electron microscopy (FE-SEM). Fig. 5a shows SEM image of graphene oxide with a smooth surface and some overlapping. Fig. 5b shows more wrinkled morphology for GN-CP nanohybrid compared to the pristine GO. The GO sheets can self-assemble after chemical functionalization with CP which result in aggregation of smaller flakes

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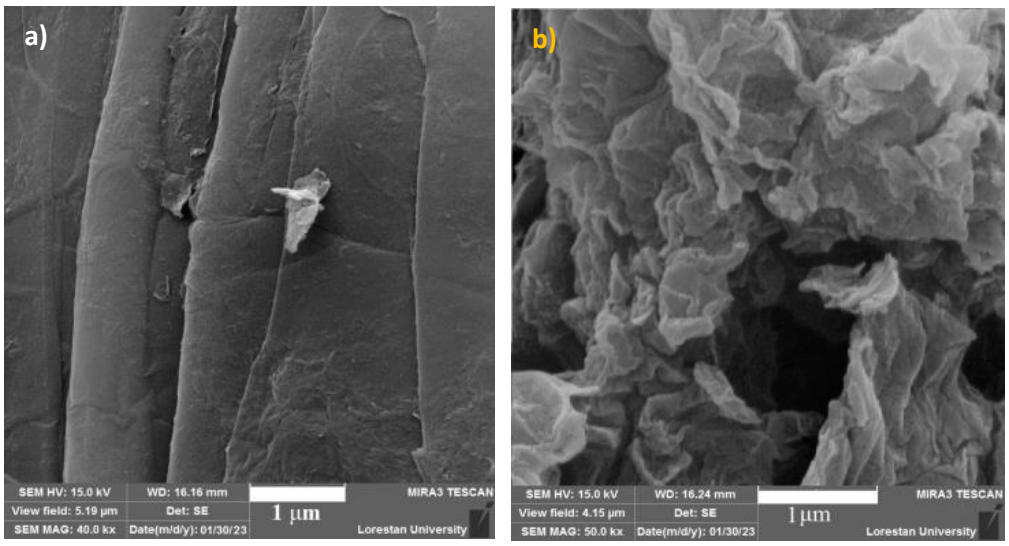


Fig. 5. FE-SEM images of a) GO and b) GN-CP.

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Antibacterial and antioxidant activities

GN, CP, and GN-CP were investigated for antibacterial activity against Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. coli*. Bacteria cells were incubated with dispersions of materials (concentration 40 µg/mL) in isotonic saline solution for 2 h under 250 rpm shaking speed. The viability of bacteria was determined by the colony counting method. The results are shown in Fig. 6 as percent loss of viability for bacteria cells. CP, which is a quaternary pyridinium compound of curcumin, exhibited the strong and weak activities against Gram-positive bacteria *S.aureus* and Gram-negative bacteria *E.coli*, respectively. This outcome showed that the existence of alkyl chain is effective on Gram-positive bacteria, but not so much on Gram-negative bacteria. GO and GN demonstrated a mild activity against both types of bacteria by the mentioned mechanisms in the introduction section. By grafting of CP on the GN, a high activity was seen against *S.aureus* and *E.coli* bacteria. The simultaneous presence of graphene nanosheets and quaternary pyridinium compound has strongly affected both types of bacteria. The bacterial cellular membrane has negative charges and the GN possesses positively charged surface area and consequently can efficiently absorb the bacteria on its nanosheets. After that, the penetration of the alkyl groups of CP into the bacterial membrane causes a deep disruption of cell wall.

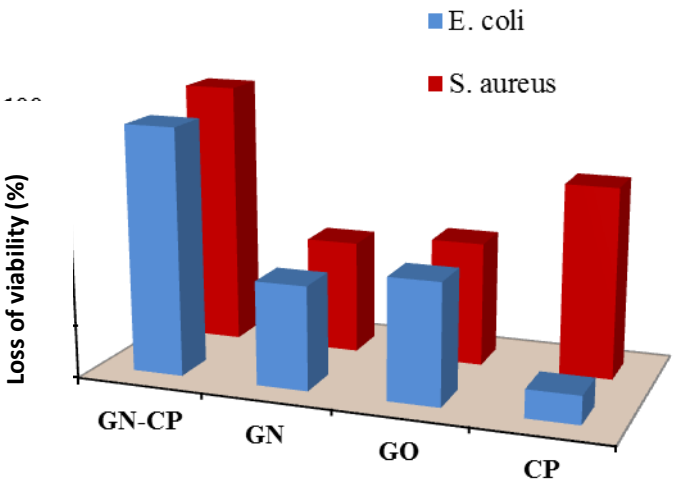


Fig. 6. The viability of *E. coli* cells and *S. aureus* cells (10^6–10^7 CFU/mL) incubating with materials suspensions (40 µg/mL) for 2 h.

Table 1. Antioxidant activity of compounds by DPPH Scavenging.

Compound	DPPH Scavenging (% ± SD, n=3) at concentration=1 mg/mL
Curcumin	98.76 ± 0.60
CP	91.38± 0.61
GO	45.30 ± 0.52
GN	34.12 ± 0.55
GN-CP	86.41 ± 0.26

The results of antioxidant activity assay as the inhibiting free radicals of DPPH were listed in table 1. Curcumin showed the highest free radical scavenging that attributed to methoxy, phenolic, and α,β-unsaturated diketone groups. CP has preserved the antioxidant activity of curcumin to a great extent. It has been shown that graphene based materials exhibit a significant antioxidant activity. GN-CP nanohybrid showed a notable and a comparable activity to CP and curcumin.

Conclusions

Synthesis of new compounds with improved medical properties is an essential issue. In present study, a curcumin/quaternary pyridinium compound was synthesized and covalently conjugated on the surface of graphene nanosheets. The hybrid material showed high antibacterial activity against *S. aureus* and *E.coli* bacteria and also, significant antioxidant activity, attributing to synergic effect of quaternary pyridinium and graphene segments. The results revealed that the new compound can be worth to further study as a promising antibacterial and antioxidant agent.

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